Seasonal Influenza Vaccine Effectiveness in Asthma

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Abstract

Introduction

Influenza is a seasonal viral respiratory infection that causes considerable morbidity and mortality, particularly in individuals with chronic medical conditions such as asthma. Vaccination is one of the most effective available preventive measures against influenza and is recommended for children and adults with asthma. Despite the longstanding recommendation in developed countries that people with asthma be vaccinated against influenza, less than half of the asthma population eligible for the vaccine are immunised every year. Some of the reasons for this suboptimal coverage include doubts about the benefits of the vaccines and safety concerns amongst both healthcare providers and asthma patients. In 2012, a Cochrane systematic review concluded that evidence from randomised controlled trials (RCTs) on the effect of influenza vaccines on asthma-related clinical outcomes from influenza infection is unclear. However, the review confirmed that influenza vaccination was safe. Therefore, this doctoral research program had the following main aims:

1. To conduct rigorous secondary research in the form of a systematic review to identify, appraise and integrate evidence, not limited by study design, for vaccine protection in asthma against influenza infection and influenza-related clinical outcomes. This review also included and appraised evidence on vaccination safety in people with asthma.
2. To conduct primary research work to fill evidence gaps identified in the systematic review by providing more accurate estimates of the protective effects of influenza vaccines against influenza infection in people with asthma over multiple influenza seasons using routinely collected data from Scotland.
3. To estimate the effectiveness of influenza vaccination in preventing laboratory-confirmed influenza in people with asthma, and variation in vaccine effectiveness (VE) between influenza seasons, influenza types/subtypes, influenza vaccine types and other asthma individuals' characteristics. To measure the vaccine uptake and explore the main characteristics of people with asthma related to influenza vaccination uptake using routinely collected data from community and secondary healthcare level settings.

Methods

A programme of work was undertaken which included three complementary phases. In the **Phase One,** I conducted a systematic review of the literature and searched for published and unpublished studies assessing the efficacy, effectiveness and safety of influenza vaccines over a 46-year period (1970 to 2016). Study selection, data extraction and quality appraisal of studies was carried out independently by two reviewers who followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) reporting checklist. Meta-analysis of clinical and epidemiological similar studies was also performed.

In **Phase Two,** I conducted a test-negative design (TND) case-control study to assess the VE against real-time polymerase chain reaction (RT-PCR) laboratory confirmed influenza in people with asthma. For this study, I used Scottish health administrative data from the Seasonal Influenza Vaccine Effectiveness II (SIVE II) project over 14 influenza seasons (2000/01 to 2015/16). The SIVE II project used healthcare data collected from routinely available datasets and created a large national primary care and laboratory-linked dataset. The main aim of the SIVE II project was to assess the effectiveness of the live attenuated and trivalent inactivated influenza vaccines of all at-risk groups for influenza (e.g. asthma) in Scotland. Some of the SIVE project's objectives were the evaluation of VE against RT-PCR laboratory-confirmed influenza and influenza-related clinical outcomes (e.g. influenza and asthma-related general practice consultations, hospitalisations and death). Therefore, my work in this phase was part of the SIVE II project as it focused on people with asthma. Additional stratified VE estimates related to various viral strains, vaccines types and asthma individuals' characteristics were also provided, but from seasons 2010/11 onwards. A generalised additive logistic regression model was used for the calculation of all the VE estimates.

Finally, in the **Phase Three**, I undertook a vaccine uptake analysis exploring factors related to vaccine uptake in the asthma population using data from primary care centres and hospitalisation over 16 influenza seasons (2000/01 to 2016/17) in Scotland. A multivariate logistic regression model was performed to identity any relation between characteristics of the asthma population and vaccine uptake levels in the community.

Results

The Systematic review identified 20,396 papers, and 35 studies met the inclusion criteria. It was possible to carry out meta-analyses of data from four of these studies. The review found that the influenza vaccination protects children and adults with asthma against influenza infection and influenza-related complications such as asthma exacerbations. However, the overall quality of evidence was rated as very low for all outcomes. The meta-analysis of two TND studies found a moderate VE of 45.0% (95% confidence interval (CI): 31.0 to 56.0) against influenza infection over two seasons in the United States (US) population. The protective effects of the vaccine against asthma exacerbations was also identified based on another US based study over a 3-year period in children with asthma. Specifically, the incidence rate ratio of the vaccine against asthma hospitalizations and emergency department visits ranged from 0.6 (95% CI: 0.4 to 0.8) to 0.8 (95% CI: 0.6 to 1.1). The safety of the inactivated vaccines was also shown. Specifically, a large RCT study in US found an absolute difference of 1.1 percent (95% CI: -1.4 to 3.6) for asthma exacerbations between 2,032 inactivated vaccine and placebo recipients. However, more evidence is needed to assess the safety of the live attenuated vaccines against asthma-related adverse events in preschool children.

The TND case-control study included 6,921 swab samples tested for influenza and identified an overall VE of 49.4% (95% CI: 39.7 to 57.5) in 5,824 asthma patients over a 14-year period in Scotland. Higher and significant VE estimates were observed in seasons with good antigenic match between the viral and vaccine strains. The highest VE (76.1%; 95% CI: 55.6 to 87.1) was found in 2010/11 season where the A(H1N1) strain dominated and a good antigenic match with the vaccine was found. Significant protection was observed against the A(H1N1) and B strains, with non-significant protection for the A(H3N2) strain. Significant VE estimates were found in younger adults aged 18-54 years old (VE: 54.0%; 95% CI: 39.2 to 65.2) where protection was found against all circulating influenza strains. The live vaccines offered protection against the influenza B type in children $\leq$ 17 years old (VE: 96.4%; 95% CI: 46.0 to 100.0).

The vaccine uptake analysis revealed characteristics in the asthma population that are related to higher uptake of the vaccine. The overall uptake was 33.6% among 194,319 individuals with asthma identified from 223 general practices and 65.9% among 6,232 patients with asthma with an emergency hospital admission due to influenza or pneumonia over a 16-year period. In the community and at hospital settings higher uptake levels were observed for females (38.7% and 37.0%), younger children aged 5-11 years old (24.5% and 0.8%), older adults aged ≥75 years old (82.8% and 31.0%) and influenza vaccination history (80.5% and 56.8%). History of primary and secondary care visits (70.4% and 52.8%) and the presence of multiple medical conditions (83.2%) were also related with higher uptake in the community. Higher vaccination rates (65.2%) were observed in individuals using medications, particularly inhaled and oral corticosteroids. Females, adults aged ≥65 years old, individuals living in remote rural areas, with comorbidities, with history of influenza or pneumococcal vaccination, ex-smokers and with history of five primary care visits and two emergency hospital admissions were more likely to have been vaccinated in the current season.

Conclusions

This program of work has identified evidence supporting the protective effects of influenza vaccination asthma from previous studies and has contributed new evidence supporting the use of influenza vaccination in the asthma population. In addition, factors related to vaccine uptake in people with asthma were explored. Thus, the current suboptimal uptake levels seen in the UK and globally might be improved by more effective targeting interventions to subgroups of people with asthma defined by specific demographic, clinical and other characteristics that are associated with a lower propensity for vaccination. The systematic review and the TND case-control study confirm that a moderate protection against laboratory diagnosed influenza infection can be achieved with current vaccines in people with asthma. Evidence from the systematic review also showed beneficial effects of the vaccination against clinical outcomes such as influenza-related asthma exacerbations and healthcare visits or hospital admissions. In addition, the primary research work provided stratified VE estimates across multiple influenza seasons and confirmed that vaccination prevents influenza incidence and complication in children and adults with asthma. This thesis

has therefore contributed to filling important gaps in the evidence base regarding the benefits of the influenza vaccination in people with asthma. It has also identified the need for additional evidence: specifically, studies should now focus on providing VE estimates for older adults (e.g. 55 years old), for children with LAIV administration and for asthma-related clinical outcomes. Vaccination policymakers can now use my research findings (for example, providing better estimates of safety and effectiveness to inform shared decision making and how to better target interventions to promote uptake) to enhance evidence-based recommendations for clinical practice that could result in improved health of people with asthma by preventing or reducing the burden of influenza virus during each influenza season.

Lay summary

Influenza, more commonly known as 'flu', is a viral infection that can affect breathing and trigger asthma attacks. Most people infected with the flu virus recover completely within a week, but sometimes flu causes severe, longer-lasting symptoms and even death. People who have asthma are more likely to get more severe forms of flu. In the UK and worldwide flu vaccination is recommended each winter for people aged six months who are at greater risk of severe flu, such as those who have asthma.

However, the benefits and possible harms from vaccination in people with asthma against flu have not been fully described. In this research, I aimed to assess how well flu vaccines protect people with asthma from flu and flu-related illness such as asthma attacks triggered by flu.

To do this, I first searched for research studies already done that assessed the benefits and possible side-effects of the flu vaccines. Then, I studied those vaccinated against flu and compared the number who sought medical help and who turned out to a positive test for flu with those who had a negative test for flu. I used the electronic medical records of children and adults with asthma from 2000 to 2015 for this. I also explored characteristics of people with asthma in relation to vaccine uptake from 2000 to 2016. Electronic medical records were also used to measure the vaccine uptake in relation to asthma individuals' characteristics such as gender, age, socioeconomic status, type of residence, smoking history, other at-risk chronic diseases, previous vaccination or healthcare use history and prescriptions.

The review of the literature found that the flu vaccines can protect people with asthma from flu illness, asthma attacks and other important consequences (e.g. having to go to your GP or hospital, respiratory illness). However, the quality of these studies that showed the benefits of flu vaccination was poor. The review showed some evidence that supported the benefits of the vaccines, but the evidence was not good enough to draw any clear conclusions. This review also found that the flu vaccines were safe and generally well tolerated in children and adults with asthma.

My research study found that the flu vaccine could prevent around half the people with asthma from getting flu each year. Higher protection was seen during seasons when

there was a good match between the vaccine and the particular kind of flu that circulated. The flu vaccine gave better protection to younger adults and against the flu types A(H1N1) and B. Finally, a live flu vaccine (nasal spray) gave very high protection against the flu B type to children with asthma compared to the inactivated vaccine (flu jab). My research work also found higher vaccination rates in people with asthma with characteristics including females, older adults, with multiple chronic diseases, non-smokers, with previous vaccination or healthcare use history and on prescribed medications.

Findings from this research add support for vaccinating people with asthma against flu each year. In addition, this research could help the flu vaccination programme improve the health of people with asthma by providing evidence to help drive uptake of the vaccine. This would help prevent or reduce the burden of the flu and flu-related complications such as asthma attacks which are triggered by the flu virus.

Declaration

I hereby declare that the thesis has been composed by me and that the work is my own, and it has not been submitted for any other degree or professional qualification.

Eleftheria Vasileiou

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Figure 5.3 from Simpson CR, et al. Evaluating the effectiveness, impact and safety of live attenuated and seasonal inactivated influenza vaccination: protocol for the Seasonal Influenza Vaccination Effectiveness II (SIVE II) study. BMJ Open 2017; 7(2): e014200

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Abbreviations

ACIP – Advisory Committee on Immunization Practices

ACOS – Asthma COPD Overlap Syndrome

AD – Anno Domini

AUK – Asthma UK

AUKCAR – Asthma UK Centre for Applied Research

BC – Before Christ

BMI – Body Mass Index

BTS – British Thoracic Society

CDSR – Cochrane Database of Systematic Reviews

CENTRAL – Cochrane Central Register of Controlled Trials

CHI – Community Health Index

CI – Confidence Interval

CINAHL – Cumulative Index to Nursing and Allied Health Literature

COPD – Chronic Obstructive Pulmonary Disease

ECDC – European Centre for Disease Prevention and Control

ECOSS – Electronic Communication of Surveillance in Scotland

ED – Emergency Department

eDRIS – electronic Data Research and Innovation Service

ELISA – Enzyme-Linked Immunosorbent Assay

EPHPP – Effective Public Health Practice Project

ESCRO – Enhanced Services Contract Reporting Options

EU – European Union

FDA – Food and Drug Administration

GINA – Global Initiative for Asthma

GISRS – Global Influenza Surveillance and Response System

GP – General Practice

GRADE – Grading of Recommendations Assessment, Development and Evaluation

HA – Hemagglutinin

HIV – Human Immunodeficiency Virus

HPS – Health Protection Scotland

ICD – International Classification of Diseases and Related Health Problems diagnostic code

ICS – Inhaled Corticosteroids

ICTRP – International Clinical Trials Registry Platform

IL-6 – Interleukin-6

IL-8 – Interleukin-8

ILI – Influenza like Illness

I-MOVE – Influenza – Monitoring Vaccine Effectiveness

IRU – Incidence Rate in Unvaccinated

IRV – Incidence Rate in Vaccinated

ISD – Information Services Division

LABA – Long Acting Beta Agonists

LAIV – Live Attenuated Influenza Vaccine

LAMA – Long Acting Muscarinic Antagonists

LRTI – Lower Respiratory Tract Infection

LTRA - Leukotriene Receptor Antagonists

NA – Neuraminidase

NHS – National Health Services

NSS – National Services Scotland

OCS – Oral Corticosteroids

OR – Odds Ratio

PCR – Polymerase Chain Reaction

PIPER – Pandemic Influenza Primary Care Reporting

PRISMA – Preferred Reporting Items for Systematic Reviews and Meta-analyses

PROSPERO – International Prospective Register of Systematic Reviews

QIV – Quadrivalent Inactivated Vaccine

RANTES – Regulated on Activation, Normal T Expressed and Secreted

RCT – Randomised Controlled Trial

RECORD - REporting of studies Conducted using Observational Routinely-collected Data

RNA – Ribonucleic Acid

RR – Risk Ratio or Relative Risk

RSV – Respiratory Syncytial Virus

RT-PCR – Reverse Transcription-Polymerase Chain Reaction

SD – Standard Deviation

SES – Socio-economic Status

SIMD – Scottish Index of Multiple Deprivation

SIRS – Scottish Immunisation & Recall System

SISRS – Scottish Influenza Surveillance Reporting Scheme

SIVE – Seasonal Influenza Vaccine Effectiveness

SMR – Scottish Morbidity Record

SPIRE – Scottish Primary Care Information Resource

SSPC – Scottish School of Primary Care

STROBE - Strengthening the Reporting of Observational studies in Epidemiology

TIV – Trivalent Inactivated Vaccine

TND – Test Negative Design

UK – United Kingdom

UR8 – Urban/Rural 8 fold classification

URTI – Upper Respiratory Tract Infection

US – United States

VE – Vaccine Effectiveness

WHO – World Health Organization

WHOLIS – World Health Organization Library Information System

WoSSVS – West of Scotland Specialist Virology Centre

Chapter 1. Introduction

This chapter includes introductory information on the disease burden of respiratory viral infections, in particular that caused by the influenza virus, in people with asthma. Influenza vaccines are currently the only available preventive immunisation measure against influenza in people with asthma. This chapter describes the available influenza vaccines, vaccination guidelines, and considerations on how best to estimate influenza vaccines effectiveness (VE). The main aims of this chapter are to summarise evidence regarding the impact of influenza infection on morbidity and mortality in people living with asthma and the evidence supporting the use of the influenza vaccines to prevent influenza in at-risk groups nationally and globally.

1.1 Respiratory viral infections in asthma

Overall, upper respiratory tract viral infections (URTI) or "common colds" account for the majority of respiratory viral infections. In adults, the most frequently isolated URT viruses are rhinoviruses (40-50%) and coronaviruses (20-30%) followed by influenza viruses (10-15%). In infants and young children (<5 years old) respiratory syncytial viruses (RSV) are the most frequent circulating viruses.(1)(2)(3)

Acute upper respiratory viral infections can induce asthma exacerbations.(4)(5) In the community, it is estimated that a respiratory viral infection is responsible for 80-85% of asthma exacerbations in children and 44% in adults.(6)(7)(8) Rhinoviruses are the most common viral triggers of asthma exacerbations accounting for 66% in children and 60% in adults.(6) In early childhood, RSV and parainfluenza viruses are the most common triggers of asthma attacks, while in older children rhinoviruses and influenza viruses are most dominant (see Figure 1.1).(7)

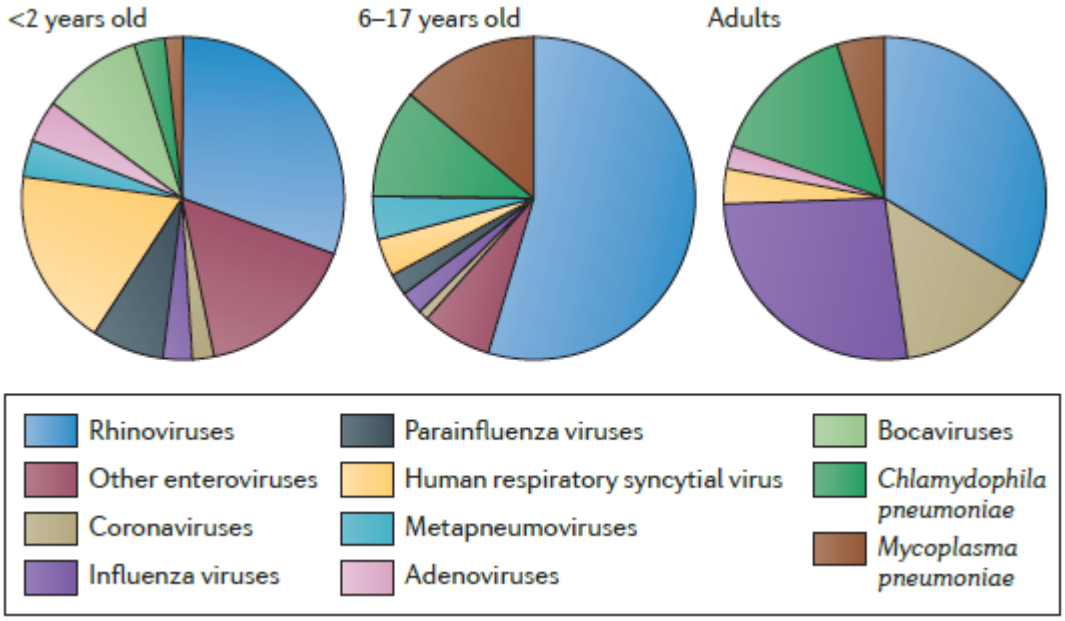

Figure 1.1: Viruses and bacteria associated with asthma exacerbations
The prevalence of viruses and bacteria in young children (<2 years old), older children (6–17 years old) and adults, presented as median percentages from several studies. Enterovirus estimations in adults and bocavirus estimations in 6–17 year olds and in adults may be under-represented since data is not available in published studies. Reproduced with permissions from: Edwards MR, Bartlett NW, Hussell T, Openshaw P, Johnston SL. The microbiology of asthma. Nat Rev Microbiol 2012; 10(7): 459-71.

Johnston and Papadopoulos (9) hypothesised that both children and adults with asthma are possibly susceptible to respiratory viral infections because:

1. *"People with asthma are more susceptible to virus infection than people without asthma"*
2. *"People with asthma manifest symptoms of respiratory infection more readily (because they wheeze) than people without asthma and thus have fewer episodes of subclinical infection"*

The above hypotheses, derived from Johnston and Papadopoulos (9), observes that children with asthma have usually more respiratory infections than their asthma-free siblings and vice versa some children with frequent respiratory infection episodes are diagnosed with asthma.

Relevant epidemiological evidence confirms the association between respiratory viral infections and asthma exacerbations. Specifically, Johnston and Papadopoulos(9) support their hypotheses based on the following biological criteria:

1. There is a seasonal variation of respiratory viruses which closely precedes that of asthma exacerbations in both children and adults with asthma.
2. A dose-response relationship has been observed between the viral infections and asthma exacerbations. Severe asthma exacerbations are more common in individuals with a severe respiratory tract viral infection.
3. The prevalence of predominant viruses for various age groups is the same for the viruses that trigger asthma exacerbations.
4. The temporal variation of asthma exacerbations follows the seasonality of most respiratory viruses and not to other environmental triggers.
5. Individuals with asthma are more likely to have had respiratory symptoms due to a viral infection which preceded asthma symptoms.

Thus, the biological causality provided by previous epidemiological and clinical studies leaves no room for doubt that respiratory viruses are linked with asthma exacerbations and are the most common environmental triggers.(9)

The role of bacterial respiratory tract infections in the pathogenesis of asthma exacerbations has also been shown in the literature.(10)(11) Most common bacterial pathogens associated with asthma exacerbations are Haemophilus influenzae, Streptococcus pneumoniae, and Moraxella catarrhalis.(12)(13)(14) A study in Japan reported that Haemophilus influenzae was observed in stable patients with asthma and other bacteria particularly Streptococcus pneumoniae was associated with asthma exacerbations in adult inpatients. Overall viral or bacterial infections were detected in 70% of inpatients with an asthma attack.(15)

Atypical bacteria such as Mycoplasma pneumoniae and Chlamydophila pneumoniae are also common respiratory pathogens linked with asthma exacerbations in children and adults with asthma.(10)(11)(15) The incidence of the atypical bacterial infections related to asthma exacerbations is higher (38.7%) in children older than five year olds compared to younger children (10.0%).(16) While, in adults studies have shown the presence of atypical bacterial infections caused by the Chlamydia pneumoniae pathogen in around 8% of asthma exacerbations.(17) The co-infection of asthma patients with bacterial and viral respiratory pathogens can lead to considerable higher

detection rates (40-60%) of these atypical bacterial pathogens during asthma exacerbations.(11)

Healthcare professionals usually test for respiratory viruses in people with acute onset of respiratory illness or in people at-risk of severe respiratory illness due to age or underlying medical conditions who develop respiratory illness. For example, clinicians should test outpatients for influenza in high-risk patients, in patients with acute onset of respiratory symptoms and in patients who present with influenza-like illness, pneumonia or nonspecific respiratory illness. Clinicians also should test inpatients such as patients hospitalised with acute respiratory illness, patients with acute deterioration of chronic underlying conditions (e.g. cardiopulmonary disease), patients admitted who are at-risk of complications and present acute respiratory symptoms and patients that while hospitalised develop acute respiratory symptoms or respiratory distress without a clear alternative diagnosis.(18)

There are various tests available to clinicians to support the diagnoses of respiratory viruses such as influenza. Specifically, rapid molecular tests (e.g. nucleic acid amplification tests) should be used in outpatients to detect influenza infection. Other molecular tests (e.g. multiplex reverse-transcription polymerase chain reaction assays) should be used in inpatients to detect influenza infection and other respiratory pathogens. Nowadays, viral culture is mainly used only to confirm any negative results from less sensitive diagnostic tests (e.g. immunofluorescence assays) and to provide isolates for further characterisation.(18) Viral culture has been for decades the gold-standard test for the detection of respiratory viruses. However, diagnosis of viral pathogens is predominantly now carried out with molecular tests such as polymerase chain reaction (PCR) assays and rapid nucleic acid assays) for faster and highly accurate detection of viral pathogens in outpatients and inpatient clinical settings.(19) Molecular tests can detect fragments of a viral pathogen without requiring the whole infectious pathogen as it is the case in viral culture. As a result, molecular tests have enabled large epidemiological studies for known and newly discovered respiratory viral pathogens, and the diagnosis of pathogens (e.g. coronaviruses and group C rhinoviruses) which cannot be detected via viral culture.(19)

In clinical practice the use of molecular tests such as PCR tests has enabled physicians to provide a more accurate and timely diagnosis and treatment of viral respiratory infections.(19) Specifically, the early diagnosis of the type of respiratory infection (e.g. viral or bacterial) ensures the correct treatment regime which reduces the replication and the transmission of the viral pathogen. For example, children will receive prompt antiviral treatment which can improve clinical outcome, reduce the inappropriate use of antibiotics and the reduce the transmission of the infection to the community.(20)

The use of PCR and other molecular tests has also improved the diagnosis of bacterial respiratory infections which are commonly associated with lower respiratory tract infections (e.g. community-acquired pneumonia).(21) Culture of specimens (e.g. sputum) for suspected bacterial infection is also used in hospitalised patients. However, detection of bacterial pathogens is less common (and is rarely carried out in primary care).(21) The use of these new diagnostic technologies provides better clinical management and has supported advancements in public health surveillance.

1.2 Overview of influenza and asthma

1.2.1 Basic concepts of influenza virus

Definition

Influenza is an acute respiratory tract infection caused by influenza viruses. Influenza viruses are single-stranded ribonucleic acid (RNA) viruses and derive from the Orthomyxoviridae family, and are classified into three categories (A, B and C) based on their core nucleoproteins which enclose the viral genome for functions such as RNA transcription, replication and packaging.(22) Influenza A virus is classified into subtypes according to two glycoproteins (hemagglutinin (HA) and neuraminidase (NA)) on its surface. There are 18 different hemagglutinin (HA) and 11 different neuraminidase (NA) subtypes for influenza A viruses. Influenza B virus is not divided into subtypes but contains one of the two lineages B/Victoria and B/Yamagata. The most common influenza A virus's subtypes circulating in humans are A(H1N1) and A(H3N2). The main reservoir of influenza A viruses is located in wild aquatic shore birds. Influenza A viruses are also found in swine, domestic poultry, horses, dogs, cats, bats, marine mammals and humans.(23) Influenza B and C viruses are found mainly

in humans. Influenza A and B have a substantial effect in humans whilst influenza C viruses only causes mild disease and does not lead to epidemics.(24)(25)

Epidemiology

Influenza viral infection is most common in young individuals including older children and young adults. However, higher morbidity and mortality rates are seen in individuals older than 65 and among those with chronic underlying conditions such as chronic respiratory diseases.(26) Initial influenza infection usually occurs in school-aged children (35-50%) before its spread to pre-school children and the elderly.(26)

According to the World Health Organization (WHO), annual influenza epidemics cause about 3-5 million cases of severe illness, and about 250,000 to 500,000 deaths globally/year.(27) While influenza-related illness is most often mild, it can be much more severe and may lead to death. Hospital admissions and deaths are most likely in individuals who are most at risk from compilations of influenza due to underlying medical conditions.(27) Individuals with certain medical conditions, specific age groups, pregnant women and health care providers are perceived to be at high risk of severe disease or complications due to influenza.(28-30) However, it should be acknowledged that although underlying mechanisms have been proposed to explain why people with these conditions should have severe disease when infected with respiratory viruses like asthma, there are few (epidemiological) studies which have explored whether these underlying conditions in the presence of influenza and in the absence of an early intervention, would indeed lead to more severe disease or death.(28-30) Further work is therefore needed to explore this proposed hypothesis.

Mutation

Influenza viruses are constantly mutating because of a characteristic of RNA genome viruses which results in variability of the HA and NA antigens. Two reasons for the high mutation rate of influenza viruses leading to rapid rates of viral evolution include: a) the viral genome of influenza is RNA-based which is considered an unstable molecule compared to DNA viral genome. In addition, RNA viruses do not have a proofreading mechanism when they replicate as DNA viruses have during their reproductive process. Thus, errors in the RNA genome happen more frequently leading to high mutations rates of the influenza viruses and b) the single-stranded segmented

RNA genome of influenza viruses means that coding sequences are located on separate strands of RNA. Thus, viral genetic material is frequently combined and re-arranged when host cells are simultaneously infected with multiple types of influenza viruses.(31)(32)

Two common types of mutations are the “antigenic drift” and the “antigenic shift”. Antigenic drift happens when minor mutations lead to small changes in the genes of influenza viruses. Small changes in HA antigens happen very often. Antigenic shift occurs when major mutations lead to substantial changes in the HA or NA antigens. Thus, new influenza A subtypes can emerge as well as new HA antigen or HA and NA antigen combinations derived from animals, resulting in different subtypes to those found in humans. The significance of antigenic shift compared to antigenic drift, is that in antigenic shift, humans will have low or no antibody memory to prevent the replication of these new influenza viruses which can lead to an influenza pandemic.(33) Antigenic shift has not been reported in influenza B viruses. The B virus does not affect species other than humans. Thus, the chance of substantial changes on its surface antigens is minimised, which further explains the absence of influenza B subtypes. However, small changes (antigenic drift) have been reported, which has resulted in co-circulation of both Victoria and Yamagata strains or so-called lineages of the influenza B virus.(24)(34)

Transmission and infection

Influenza viruses can be transmitted from animals to humans (rare), or between humans (very common). The transmission from animals to humans can occur through direct contact with infected animals or their by-products. Additionally, people can be infected from animals through interaction with environmental sources contaminated with influenza viruses (e.g. water sources, faeces, feathers and surfaces).(35) Transmission between humans occurs via droplets aerosol when someone with influenza infection coughs, sneezes or talks. The transmission in humans occurs via three main routes including droplets, droplet nuclei (or aerosols) and contact.(36-39)Droplets are large particles (>10μm) that are produced when someone coughs or sneezes and travel only a short distance (≤1m). Droplets usually reach the upper respiratory tract such as mouth or nose but not the lungs due to their large size. Droplet

nuclei or aerosols are small particles (<5 μm) that can be inhaled due to their small size. Thus, they can reach the lower respiratory tract and also deposit on the upper respiratory tract.(38)(39) Contact transmission occurs directly when respiratory particles are transferred to another person's mucous membrane of the upper respiratory tract or indirectly when someone touches a contaminated object or person.(37)(39)

The incubation period which refers to the time between the exposure to the influenza virus and the onset of the first influenza symptoms usually starts one day before the onset of symptoms and last five to seven days after the onset of symptoms. Symptom onset is usually sudden. In children, symptoms can last more than seven days, while in immunocompromised individuals the symptoms can persist for weeks or months.(37)(38)

Clinical features

The symptomatology onset of influenza is more rapid compared to other respiratory viral infections. Common respiratory and systemic signs and symptoms of influenza include: 1) fever or feverishness; 2) non-productive cough; 3) headache; 4) sore throat; 5) runny or stuffy nose; 6) muscle or body aches; and 7) fatigue, weakness. Children are also more likely to have gastrointestinal symptoms such as vomiting and diarrhoea compared to adults.(40-42)However, people infected with influenza can also be asymptomatic. Serological studies have shown that around 30% to 50% of influenza infections in healthcare providers were asymptomatic.(36) Most of these common influenza signs and symptoms are resolved in a few days to less than two weeks. Some people will also develop complications due to influenza or secondary infection (usually bacterial pneumonia).(41) Respiratory and non-respiratory complications include abnormalities of middle ear pressure and acute otitis media (most common in children), primary viral and secondary bacterial pneumonia, myocarditis, viral encephalitis, post-infective immune-mediated encephalomyelitis, meningitis, meningoencephalitis and Guillain-Barre syndrome. People with certain medical conditions (such as lung disease, cardiovascular disease, metabolic disease, hepatic disease, kidney disease, neurologic disease, immunocompromised individuals, haematologic conditions, morbid obesity and genetic conditions) and pregnant women will also experience influenza complications related to their medical

condition.(24)(36)(41-43) In addition, children and adults can experience severe signs and symptoms. Specifically, children may develop fast breathing or trouble breathing, bluish lips or face, ribs pulling in with each breath, chest pain, severe muscle pain, dehydration, not alert or interacting when awake, seizures, high fever 40°C, no fever in children <12 weeks old, fever or cough that improves but then return or worsen and worsening of chronic medical conditions. In adults, severe signs include difficulty in breathing or shortness of breath, persistent pain or pressure in the chest or abdomen, persistent dizziness, confusion or inability to arouse, seizures, not urinating, severe muscle pain, severe weakness or unsteadiness, fever or cough that improve but then return or worsen and worsening of chronic medical conditions.(42)

Invasion and replication in host cells

Tracheobronchial epithelial cells are the initial host cells: the influenza virus then spreads to the upper and/or lower respiratory tract.(44) The virus uses glycoproteins HA on its surface to enter into the host cell in an endosome. Viral RNA is then released in the cytoplasm of the host cell and enters the cell nucleus where transcription and replication of the viral genome takes place. The viral nucleic acid leaves the nucleus and uses the host cell's plasma membrane in order to create all the necessary viral particles to exit the infected host cell and infect other neighbouring cells (see Figure 1.2).(45)

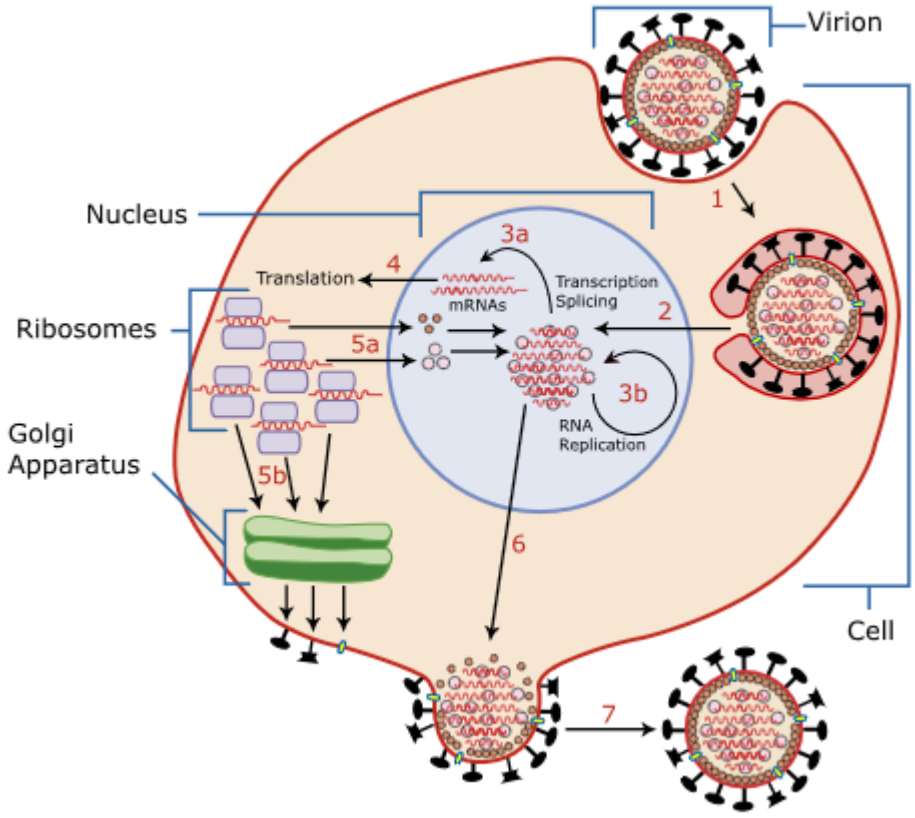

Figure 1.2: Invasion and replication of influenza virus in a host cell
Step 1: A virion attaches to the host cell membrane via HA and enters the cytoplasm by receptor-mediated endocytosis and forms an endosome. A cellular trypsin-like enzyme cleaves HA into products HA1 and HA2 (not shown). HA2 promotes fusion of the virus envelope and the endosome membranes. A minor virus envelope protein M2 acts as an ion channel thereby making the inside of the virion more acidic.
Step 2: As a result, the major envelope protein M1 dissociates from the nucleocapsid and vRNPs are translocated into the nucleus via interaction between NP and cellular transport machinery.
Step 3: In the nucleus, the viral polymerase complexes transcribe (STEP 3a) and replicate (STEP 3b) the vRNAs.
Step 4: Newly synthesized mRNAs migrate to cytoplasm where they are translated.
Step 5: Posttranslational processing of HA, NA, and M2 includes transportation via Golgi apparatus to the cell membrane (STEP 5b). NP, M1, NS1 (non-structural regulatory protein - not shown) and NEP (nuclear export protein, a minor virion component - not shown) move to the nucleus (STEP 5a) where they bind freshly synthesized copies of vRNAs.
Step 6: The newly formed nucleocapsids migrate into the cytoplasm in a NEP-dependent process and eventually interact via M1 with a region of the cell membrane where HA, NA and M2 have been inserted.
Step 7: Then the newly synthesized virions bud from infected cell. NA destroys the sialic acid moiety of cellular receptors, thereby releasing the progeny virions.)

Diagnosis

Influenza infection can be diagnosed based on either clinical signs and symptoms alone or in combination or laboratory tests. Health professionals can diagnose influenza like illness (ILI) based on clinical criteria such as fever or feverishness, malaise, headache,

myalgia, cough, sore throat and shortness of breath. An individual meeting criteria for ILI may or may not have true influenza infection, as the ILI syndrome can be caused by various respiratory viruses. Thus, a diagnosis based only on clinical symptoms cannot confirm an influenza infection and further testing is required to distinguish ILI from true influenza infection.(46-48) According to the newly revised ILI clinical case definition by the WHO an ILI is "an acute respiratory illness with a measured temperature of ≥ 38 °C and cough, with onset within the past 10 days".(49) However, there is heterogeneity of clinical cases of the ILI definition across countries. While no established definition exists in other countries (e.g. England and Scotland).(50) Although there should be little to no effect on VE estimates on the variability of these ILI definitions. For example a lower threshold for ILI (less severe) will lead to a higher number of possible test positive cases and negative for influenza controls - as long as both symptomatic vaccinated and unvaccinated individuals are tested, VE should be unaffected by ILI definition. The following diagnostic tests are used to confirm influenza infection: (51)(52)

1) Reverse Transcription-Polymerase Chain Reaction (RT-PCR) (conventional gel-based PCR, real-time RT-PCR and multiplex PCR) is a nucleic acid amplification test, which can detect viral RNA in respiratory samples with high sensitivity and specificity. The PCR tests are used for virological surveillance (e.g. annual surveillance of influenza activity) and for diagnostic purposes in patients with suspected influenza infection.(53) Healthcare professionals collect specimens from the upper and lower respiratory tract (e.g. nasopharyngeal swabs, throat swabs, nasopharyngeal or bronchial wash, nasal or endotracheal aspirate and sputum) of outpatients or inpatients with suspected influenza. The PCR tests can detect influenza types, subtypes and lineages. Results are available between 1 to 8 hours after the submission of the specimen.(54)
2) Immunofluorescence (direct or indirect antibody staining) is an antigen-detection test used for the detection of virus-infected cells in original clinical specimens and field isolates. It is a rapid test for the detection of respiratory viruses within 2 to 4 hours. It has moderate sensitivity and specificity, which is dependent on laboratory expertise and the quality of the specimen collected. Clinical specimens can be

derived from nasal or throat swabs, nasopharyngeal aspirates, nasal or throat wash, transtracheal aspirates and bronchoalveolar lavage fluids.(51)

3) Rapid Point of Care Tests (POCTs) are rapid diagnostic tests which are using nucleic acid amplification technologies (NAAT). The aim of these tests is to help the physician to determine the best respiratory care options in an accurate and timely manner. Healthcare professionals perform rapid POCTs in emergency departments, medical admission units and outpatient settings. Healthcare professionals take upper respiratory tract specimens such as nose or throat swabs, nasopharyngeal aspirates, nasal washes and sputum. The POCTs use RT-PCR or similar technology to detect influenza A and B types including specific influenza A subtypes. The results of the POCTs are available within 15-90 minutes. The sensitivity and specificity of these tests is high ranging from 90-95%.(55)
4) Viral culture (shell vial culture, isolation in cell culture) is not a screening test. However, it can be used during periods with low influenza activity (e.g., pre-influenza, post-influenza and non-influenza seasons). Additionally, it can be performed to confirm negative test results from rapid influenza diagnostic tests or immunofluorescence and for public health surveillance purposes. Viral culture has moderately high sensitivity and the highest specificity. The results are available in 2-10 days.
5) Serologic tests (hemagglutinin inhibition, enzyme-linked immunosorbent assay (ELISA), complement-fixation, and neutralisation) are only available in reference laboratories. The detection of acute influenza infection is difficult because a high increase of antibody titres is required (e.g., four-fold increase or higher). However, they are useful for epidemiological and immunological studies and for the assessment of the antibody response following influenza vaccination. They can also be used for retrospective diagnosis when the influenza virus cannot be detected – for example, when the virus stops shedding.

The clinical or laboratory diagnosis of influenza can also be challenging in the case of asymptomatic ILI. A high number of individuals with influenza infection have subclinical or asymptomatic influenza. Thus, surveillance data are unlikely to capture these individuals when they measure the annual burden and severity of influenza based

on people that develop ILI symptoms and seek care.(56) In addition, the use of molecular tests such as PCR tests may fail to detect influenza in asymptomatic individuals. A British study used PCR tests to detect influenza and other respiratory viruses in nasal swab samples and serology test to estimate antibody levels against influenza in serum. The study found that only 25% of individuals with a serologic confirmed influenza infection had also a PCR confirmed infection. Thus, 75% of individuals were asymptomatic and PCR tests failed to detect them.(56) Older serologic studies have also found that between 30 to 50% of healthcare providers were asymptomatic for influenza.(36) The absence of symptoms in infected individuals could indicate protection from past exposures to influenza infection or vaccination or older age. Studies have also found individuals with confirmed influenza infection but no serologic confirmed infection. Therefore, the use of molecular and serology tests could increase the detection of infected individuals with or without ILI symptoms.(57)

1.2.2 Basic concepts of asthma

Definition

According to the updated 2018 Global Initiative for Asthma (GINA) report, asthma is: "*Asthma is a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory airflow limitation*."(58)

The definition of asthma has evolved over time. In 2014 there was a major revision of the GINA report which included a new definition of asthma. Specifically, GINA recognised that asthma is a heterogeneous disease with chronic airway inflammation.(59) Asthma is no longer a single disease but it is now considered a heterogeneous disease or an umbrella term with different asthma subtypes (e.g. allergic asthma, non-allergic asthma, late-onset asthma, asthma with fixed airflow limitation, asthma with obesity).(58-60)

Epidemiology

Asthma is globally one of the most common chronic respiratory diseases. In 2004 Masoli et al estimated that 300 million people have asthma worldwide.(61) Estimates suggest that another 100 million people will develop asthma by 2025.(61) The global increase in asthma prevalence was also confirmed in a recent systematic review where

358 million cases of asthma were estimated in 2015.(62) However, according to Masoli et al, the true prevalence of clinical asthma cannot be estimated for several reasons such as lack of a unique asthma diagnostic test, lack of a single classification system for asthma, varying interpretation of asthma symptoms by country and the influence of increasing public and professional awareness of asthma.(61) In 2004, Masoli M et al published a report about the global burden of asthma. According to the report prevalence rates were higher in Europe (with the highest prevalence of 18.4% in Scotland), North and Latin America, Australia and New Zealand (see Figure 1.3).(61)

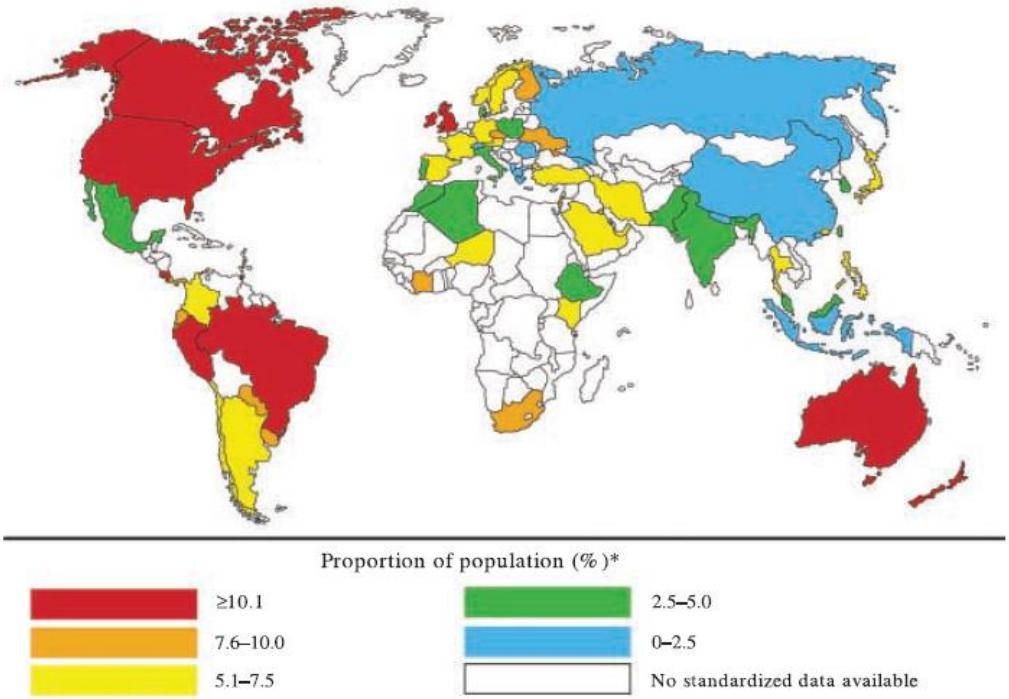

Figure 1.3: Global prevalence of clinical asthma
Reproduced with permissions from: Masoli M, et al. The global burden of asthma: executive summary of the GINA Dissemination Committee report. Allergy 2004; 59(5): 469-78.

Masoli et al used a self-reported written questionnaire about wheezing symptoms in the previous 12 months.(61) However, wheezing is not a symptom exclusively present in asthma. In addition, the diagnosis of asthma is based on a combination of asthma symptoms, physical and physiological examinations and response to asthma medication over a follow-up period. Therefore, the presence of wheezing symptoms is not equivalent to asthma diagnosis. In addition, the questionnaire was predominantly

completed by urban populations where according to the study authors the prevalence of asthma symptoms is higher in the urban then in rural populations.(61) These study limitations could have led to ascertainment bias (due to false positive cases) and overestimation of the true global asthma prevalence.(61)

Aetiology

The aetiology of asthma is not yet fully understood; however, a combination of host and environmental risk factors has been found to contribute to asthma development.(63) Common host risk factors include: genetic (e.g. genes predisposing to atopy, airway hyper-responsiveness, airway inflammation), obesity and gender. Common environmental risk factors include: allergens (indoor and outdoor), occupational sensitisers and allergens, infections (mostly viral), microbiome, tobacco smoke (passive and active smoking), air pollution (outdoor or indoor), diet.(63)(64) Certain drugs such as the anti-inflammatory medications (e.g. acetaminophen) and stress have also been reported as risk factors for asthma development. Studies have found association between acetaminophen use and children and development of asthma. Factors other than acetaminophen may have also confounded this relationship.(65) The relationship between biological, individual, family, and community-level psychosocial stress factors has also been mentioned in the literature. Nevertheless, the impact of stress on asthma development is still not fully explored.(66)(67) Therefore, more studies are needed for establish any causal link between medication and stress as risk factors of asthma.

Pathophysiology

In terms of the pathophysiology of asthma the key feature is persistent chronic inflammation and remodelling of the airways. The inflammation affects all airways, but its major physiological effects are found in medium-sized airways.(64)(68) The airway inflammation involves multiple inflammatory cells (e.g. mucosal mast cells, eosinophils, T lymphocytes, dendritic cells, macrophages and neutrophils), structural cells (airway epithelial cells, airway smooth muscle cells, endothelial cells, fibroblasts and myofibroblasts and airway nerves) and over 100 different cellular mediators, which mediate the inflammatory response.(64)

Structural changes in the asthmatic airway epithelium are also observed, often described as “airway remodelling”. The main features of airway remodelling in asthma include subepithelial fibrosis (collagen fibres and proteoglycans depose under the basement membrane of the airway wall), increased airway smooth muscle (thickness of the airway wall due to increased size and number of smooth muscle cells), increased blood vessels in airway walls (abnormal formation of blood vessels, which increase the influence of growth factors and lead to increased airway wall thickness) and mucus hypersecretion (increase of the number and size of mucous glands in the airway epithelium).(64)(68)

The persistent airway inflammation and ongoing structural remodelling leads to a common functional disorder of asthma reported as “airway hyper-responsiveness”. Airway narrowing is usually the result of this bronchial hyper-reactivity which leads to variable airflow limitation and asthma symptoms.(64) All changes increase the resistance on airways and result to decreased lung function as it has been seen in patients with chronic asthma.(68)

Clinical features

Asthma signs and symptoms vary from patient to patient; the most common features include: Nocturnal and exercised induced breathlessness, chest tightness or pain, wheezing (a whistling sound when exhaling) and coughing.(69)

The sudden or gradual over a few days deterioration of asthma symptoms can lead to the common termed as asthma attack including signs such as sever and constant wheezing, coughing and chest tightness, severe breathlessness decreasing the ability to eat, speak or even sleep, fast breathing, rapid heartbeat, drowsiness, exhaustion, dizziness, blue lips or fingers and fainting.(70)

1.2.3 Pathology of influenza virus in the asthmatic lung epithelium

The risk of severe lower tract viral respiratory infection and subsequent exacerbation is higher in patients with asthma because of impaired airway epithelium and antiviral immune responses.(71)(72) Defective mucosal antibody and antiviral responses have also been observed during an influenza infection.(73)(74) In asthma patients,

respiratory viruses such as influenza can influence all the key elements that characterise acute asthma including airway inflammation, bronchoconstriction and remodelling through a combination of multiple cellular and molecular mechanisms leading to severe asthma exacerbations.(75)(76)

Inflammation of the airway epithelium is one of the main characteristics of asthma, which involves a chain of events that can be triggered by an external stimuli such respiratory viruses and lead to an acute asthma exacerbation. Direct contact of the virus with airway epithelium cell lining triggers the production of cytokines and chemokines (proteins which are secreted by certain cells of the immune system and have an effect on other cells), which are key mediators of the inflammation response. Influenza viruses in particular stimulate the production of the cytokines interleukin-6 (IL-6), interleukin-8 (IL-8), regulated on activation, normal T expressed and secreted (RANTES) and eotaxin in the airway epithelial cells.(77)(78) Resident inflammatory cells including macrophages and lymphocytes are also activated and produce inflammatory mediators (similar to those produced by the epithelium) as a response to virus invasion and replication in them or by the local cytokine environment. The airway inflammation is further mediated by the recruitment and activation of other inflammation cells such as macrophages, eosinophils, neutrophils, mast cells and lymphocytes, which in turn produce of a wide range of inflammatory mediators.(75)(79)

The virus-induced death of the airway epithelium cells can also irritate the underlying neural tissue and modify the neural control of the airways. The presence of inflammatory products, allergens or other stimuli in the area may induce the production of broncho-constricting neuropeptides from the stimulated neural cells. Viral infection also modifies the neural mechanisms related to bronchoconstriction. Specifically, cholinergic fibres from vagal parasympathetic nerves control the neural responses in the airways. The release of acetylcholine (a Broncho-constricting neuropeptide) from vagal parasympathetic nerves is regulated by the M_2 muscarinic receptors of the neural system located in the airways through a negative feedback mechanism. However, the function of the M_2 muscarinic receptors is impaired after a

viral infection. As a result, acetylcholine is released from vagal parasympathetic nerves and binds to the M_3 muscarinic receptors on the airway smooth muscle cells resulting to smooth muscle contraction and airway bronchoconstriction (see Figure 1.4).(75)(76)(79)

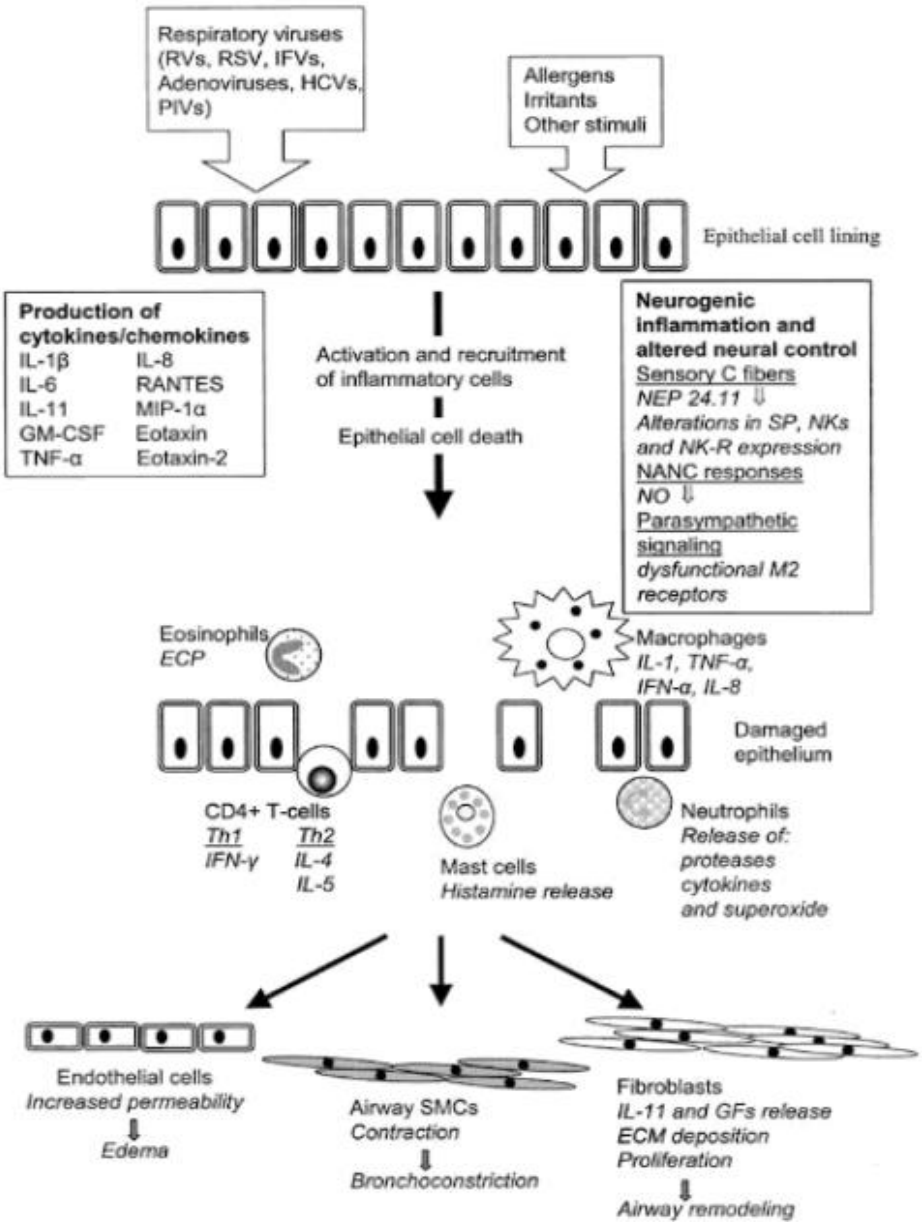

Figure 1.4: Main cellular and molecular interactions related to viral infection of the airway epithelium
Reproduced with permissions from: Johnston SL, et al. Respiratory Infections in Allergy and Asthma. New York: Marcel Dekker, Inc; 2003. 120 p.

1.3 Epidemiology of influenza virus infection in asthma

This section describes the epidemiological evidence regarding the impact of influenza virus infections in triggering asthma exacerbations and other related complications and summarises the results from key relevant studies in a critical manner.

The studies summarised in Table 1.1 provide evidence regarding the burden of influenza infections in asthma patients due to attacks and other severe complications. However, these studies vary in quality, and estimates of the disease burden are uncertain because of potentially studying non-representative populations.

The 'Manual for Estimating Disease Burden Associated with Seasonal Influenza' created by the WHO has been used to critically appraise the studies in Table 1.1.(80) According to the WHO's manual, the quality of the studies can be assessed by examining the following:

- Number of patients with asthma recruited
- Number of episodes studied
- Role of chance
- Role of bias such as selection and misclassification bias
- Effect of healthcare seeking behaviour (especially for low-income countries)
- Pattern of each influenza season and period over which the study was carried out
- Effect of sensitivity of case definition.

The studies summarised in Table 1.1 have been divided into those assessing the burden of influenza infection during non-pandemic years and pandemic years. According to the WHO Global Influenza Surveillance Network an influenza pandemic is defined as *"worldwide outbreaks of influenza caused by influenza A viruses that have undergone antigenic shift. However, as recently demonstrated, an antigenically novel virus of an existing subtype is capable of pandemic spread"*.(51)

Therefore, a pandemic year occurs when new influenza viruses emerge and spread very quickly across countries because most people do not have immunity or there is no a widely available influenza vaccine.(81) Most studies came from temperate

countries (geographical zones that span between the polar and tropic regions of earth and present a wide range of temperatures during a calendar year) where influenza follows a seasonal epidemic pattern. However, in pandemic years the influenza virus does not follow a seasonal circulation pattern as evidenced by the latest influenza A(H1N1) pandemic in 2009.(81) Characteristics' of the studies that will aid the critical interpretation of the findings are summarised in Table 1.1

1.3.1 Seasonal influenza virus infection

Studies in children

Studies involving children have been most frequently reported over the past four decades, due to the increase of asthma prevalence over this period (82) and the more recent use of highly sensitive molecular diagnostic methods such as the PCR test since late 1990's.(82)

A community based longitudinal study in the United Kingdom (UK) (1989-90) involving 108 children (aged 9-11 years old) detected only 21 (7.2%) influenza virus infections out of 292 reported respiratory episodes by either the children or their parents.(83) However, the detection of influenza was based on direct immunofluorescence (a diagnostic method with low sensitivity), while PCR was used for the detection of rhinoviruses and enteroviruses and samples from asymptomatic children. An underestimation of influenza burden is therefore possible due to misclassification bias resulting by the false negative influenza cases.(83) In contrast, a study conducted in United States (US) (1971-72) in children aged 3-11 years old, despite its small size (n=16) is still useful, because it used surveillance methods and thus the data and samples for microbiological analyses were prospectively collected.(84) Influenza-related severe symptomatic respiratory infections followed by an asthma attack were found in 10% of the children. However, the influenza-related episodes were not clearly outlined and the involvement of other pathogens when the episode was well delineated could have hampered the true detection of the influenza virus.(84) The authors of the previous study (84) conducted again the same study but this time compared the number of viral respiratory infections between children with asthma and their healthy siblings.(85) Children with asthma experienced 54 episodes of viral respiratory infections compared to the 35 viral infection episodes seen in their

siblings. In addition, influenza viruses were observed in 23% (3/13) of severe symptomatic respiratory infections in children with asthma.(85) However, the frequency of influenza infection was similar between the children with asthma and their siblings (6 vs 4). In children with asthma, the low influenza infection rate (6/54) of all viral respiratory infections and similar to that of their healthy siblings could be attributed to the small sample sizeand the inclusion of a single influenza season.

A two-year study in US assessed prospectively the association of respiratory infections and episodes of wheezing exacerbation in 32 (aged 1-5 years old) hospitalised children with asthma.(86) Only one influenza infection (4%) was related with wheezing episode in the second year. However, more than half of the children were less than three years old and RSV was the predominant circulating virus in young children, which was also confirmed in this study. In addition, the use of a serological test for the detection of influenza viruses and the high influenza vaccine coverage against the influenza A in the second year of the study children could have influenced the detection of the virus.(86)

A large (194,725 person-years) longitudinal study in US estimated the influenza burden in children (aged less than 15 years old) in relation to hospitalisations, outpatient visits and antibiotic prescriptions from 1973 to 1993.(87) The number of influenza-related complications in children with asthma was higher (incidence of influenza-related outpatient visits and antibiotic courses were 190 and 148, respectively) during all seasons compared to children with other respiratory condition (incidence of influenza-related outpatient visits and antibiotic courses were 172 and 121, respectively). However, the inclusion of children with medically treated asthma only (at least two asthma prescriptions in the previous year) yielded a low asthma prevalence (3%) and the use of conventional virology (viral culture) for the influenza detection could have underestimated the association between influenza and related complications in children with asthma. Nonetheless, the long follow-up period (20 years) of the study enabled the estimation of the influenza burden.(87)

A population-based study assessed prospectively the number of hospitalisations and outpatients visits due to influenza during four influenza seasons in 81 children (aged 6 to 59 months old) in 3 US counties.(88) The number of influenza-attributable healthcare use was higher in children with asthma compared to their healthy controls (see Figures 1.5 and 1.6). The use RT-PCR in combination of viral culture could have aid the detection of influenza viruses compared to previous studies restricted to conventional and less sensitive diagnostic tests. However, the diagnosis of asthma was based on parental reports and was not validated through a medical chart review. In addition, the diagnosis of asthma for children younger than five years is difficult and it could have also been misdiagnosed in this study leading to an overestimation of influenza-burden in the asthma group.(88)

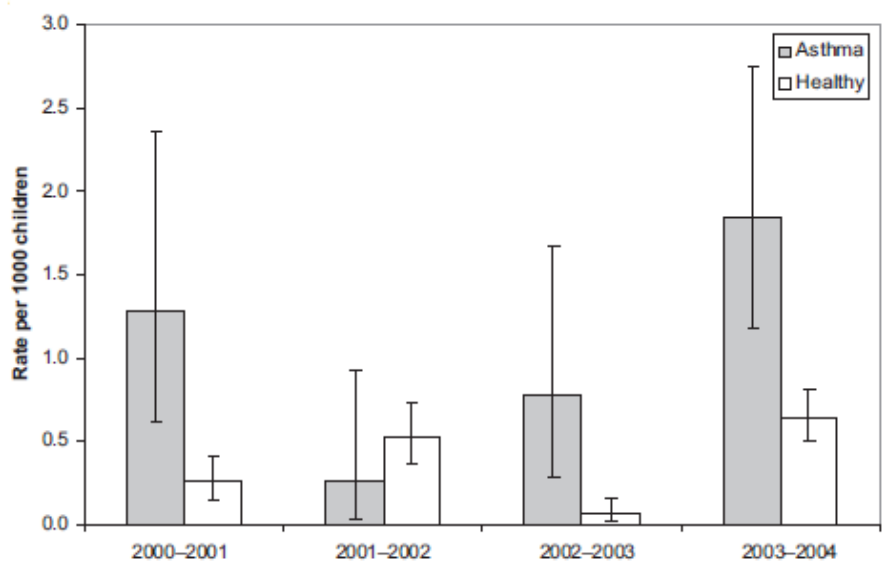

Figure 1.5: Annual influenza-attributable hospitalisation rates, with 95% CIs, for children with asthma and healthy children 6 to 59 months of age
Reproduced with permissions from: Miller EK, et al. Influenza burden for children with asthma. Pediatrics 2008; 121(1): 1-8.

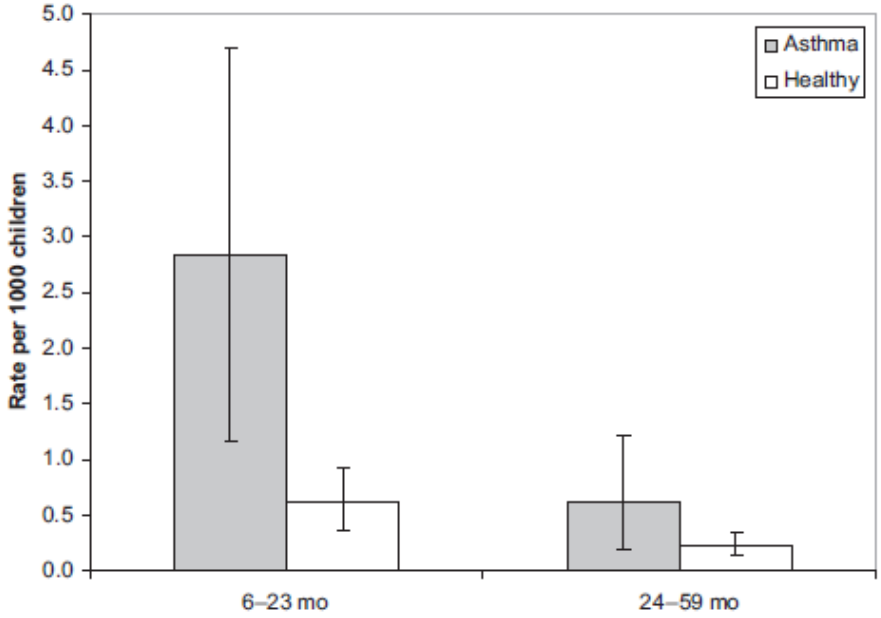

Figure 1.6: Average influenza-attributable hospitalisation rates, for 2000-2004, with 95% CIs, for children with asthma and healthy children according to age group
Reproduced with permissions from: Miller EK, et al. Influenza burden for children with asthma. Pediatrics 2008; 121(1): 1-8.

Two surveillance studies covered 12 influenza seasons, and found between 12% and 16% of children with influenza-related complications such as hospitalisations, outpatient visits and deaths had an asthma diagnosis.(89)(90) However, these studies compared the burden of influenza in at-risk children rather than explicitly focusing on asthma. Thus, the interpretation of these results is not clear for the asthma risk group.(89)(90)

A prospective study in France highlighted the underestimation of influenza infection in childhood asthma attacks during four influenza seasons.(91) The burden of influenza was evaluated in 232 hospitalised and 107 ambulatory-treated (out-patient) children with an asthma attack. Influenza A was isolated in 2.6% of asthma attacks in hospitalised children, but in 14.1% of asthma attacks in ambulatory-treated children. However, the performance of viral detection for influenza and other viruses only in hospitalised cases could have underestimated the burden of influenza by not including cases with an asthma exacerbation not requiring hospital attendance.(91) In contrast, a cross-sectional study in Korea found a high prevalence of influenza (25%) in 309 children hospitalised with an asthma attack during one year.(92) Nonetheless, only

children with severe lower respiratory tract illness requiring a hospital admission were included in this study. Critically ill young children are more likely to have a positive test for influenza, especially when highly sensitive tests such as the multiplex RT-PCR used in this study are performed.(92)

A review by Papadopoulos et al in 2011 assessed the association of respiratory viral and bacterial infections including influenza with acute asthma exacerbations.(93) According to the review, the prevalence of influenza-related acute asthma exacerbations was low in children at between 0-7%, however, an influenza-related asthma attack in children is more likely to lead to a hospital admission and healthcare utilisation.(93) The aim of the review was however to summarise up-to-date knowledge and developments in infection epidemiology in relation to acute asthma exacerbations rather than to identify all available evidence.(93) Therefore (given the aim of this review), eligible studies in influenza could have been omitted.

Studies in adolescents and adults

There were fewer studies assessing the burden of influenza infection and its complications among late childhood and young adults. The review by Papadopoulos et al also reported that the number of patients with asthma exacerbations requiring emergency or hospital admission due to an influenza infection was highest in adults, and ranged from 20 to 25%.(93)

A longitudinal study in New Zealand included asthma patients (aged 15-56 years old) and found only two influenza infections (11%) in outpatients with an asthma exacerbation during one influenza season.(94) However, the small sample size of the study (n=31) and the low circulation of the influenza virus during the study period increased the uncertainty of this finding. However, influenza virus was detected in 20% of patients with a severe asthma exacerbation.(94) Another prospective study in Australia detected influenza in 19% of hospitalised patients for an asthma attack during a 12 month period.(95) The use of viral culture and especially the serology test could have led to underestimation of influenza infections because a number of patients did not return their convalescent serum sample increasing the likelihood of undetected viral infections.(95) On the contrary, a larger study (n=138) with patients from

multiple sites in the UK during a two-year period detected only one influenza infection (1.6%) in patients with an asthma attack.(96) The use of complement fixation tests instead of immunofluorescence or enzyme immunoassay could have decreased the ability of the detection of pathogens such as influenza viruses. This is probably due to decreased viral shedding seen in adults resulting in lower virus isolation rates. Nonetheless, influenza was still associated with the most severe asthma attacks.(96)

1.3.2 Pandemic influenza virus infection

The focus of this thesis was on seasonal influenza and not on pandemic influenza. However, years with seasonal influenza activity may not reveal the true burden of the influenza in the asthma population. Some of the reasons for this could be the variability in influenza activity year by year. For example, in seasons with low viral activity healthcare providers may be more likely to report an influenza-related complication (e.g. pneumonia) as the reason for a medical attendance rather than influenza. Thus, the true burden of influenza could be underestimated. On the other hand, in seasonal with high viral activity laboratory tests may be more likely to be taken (for either surveillance or diagnostic purposes) from individuals with known high risk for influenza disease compared to the general population. In this case the disease burden of influenza will be overestimated in these at-risk groups such as asthma. Therefore, the presence of evidence on influenza burden from seasonal years only may fail to reveal the actual susceptibility of individuals with asthma to severe influenza disease. On the contrary, in pandemic years there is a plateau of high viral activity for a prolonged time which affects at-risk groups but also healthy population. Therefore, comparisons in terms of influenza burden between subgroups of the population such as asthma versus healthy individuals are feasible. Subsequently, these comparisons should reflect the actual vulnerability of individuals with asthma.

Influenza pandemics occur due to antigenic mutations in sub-types of the virus generating a novel influenza virus strain. Pandemics usually extend beyond the winter months and can spread from a focus population within weeks or months globally increasing the morbidity and mortality rates beyond the usual seasonal epidemic levels.(26)

The following four influenza pandemics have occurred during the 20th century: Spanish influenza in 1918, Asian influenza in 1957, Hong-Kong influenza in 1968 and the Russian influenza in 1977. The Spanish influenza or "flu" was the most virulent, accounting for more than 20 million deaths globally.(26)

In 2009, the swine-origin influenza A(H1N1) virus marked the first influenza pandemic of the 21st century. The combination of gene segments between avian, swine and human viruses lead to the generation of a new A(H1N1) strain that had not circulated before in human or swine (Figure 1.7). Asthma was the most common co-morbidity amongst those hospitalised due to A(H1N1)pdm09 influenza worldwide. Influenza-related asthma attacks were high in both children and adults with asthma.(97)

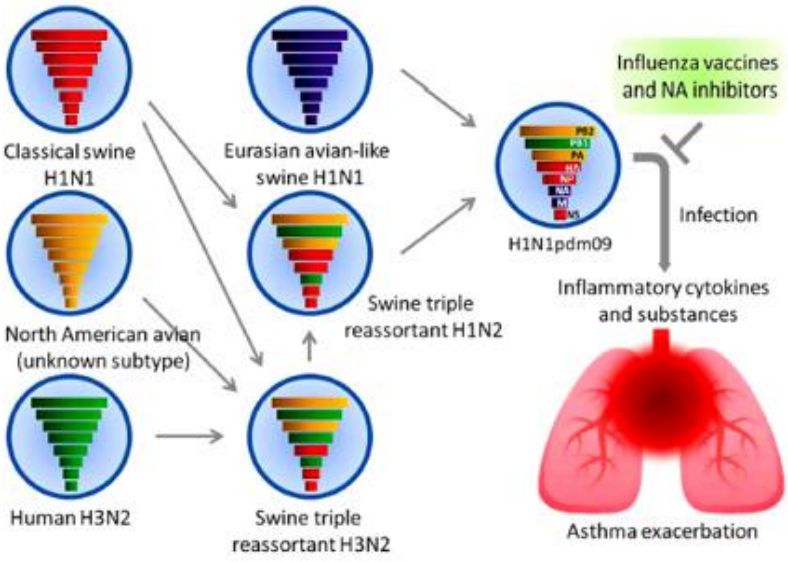

Figure 1.7: A(H1N1) pdm09 virus and asthma exacerbations
Reproduced with permissions from: Obuchi M, et al. Influenza A(H1N1)pdm09 virus and asthma. Front Microbiol 2013; 4: 307.

Four observational studies in US and in the UK assessed the number of A(H1N1)pdm infection in ambulatory and hospitalised patients. Asthma was the most frequent medical condition ranging from 25-54%.(98-101)

A US retrospective study including 5.3 million children found asthma as the most common medical condition in hospitalised children during the 2009 influenza

pandemic.(102) In addition, influenza-associated intensive care and secondary pneumonia rates were 6% higher during the 2009 pandemic compared to previous seasons. However, clinicians were testing more intensively for influenza in hospitalised patients during the pandemic and increase the use of RT-PCR than in previous seasons increasing the likelihood of an influenza diagnosis. Data on asthma severity were also not available which could have explained the reasons of hospital or intensive care unit admissions other than influenza-related asthma attacks.(102)

Another retrospective study also assessed the association between 2009 pandemic influenza and severe outcomes in asthma inpatients, but in comparison to non-asthma controls (n=1,520) across 75 UK hospitals.(103) Patients with asthma were more likely to require oxygen support during their hospital admission than non-asthma patients (36.4% vs 26.0%, unadjusted Odds Ratio (OR): 1.63, 95% confidence interval (CI): 1.27 to 2.08). However, pneumonia rates were similar between asthma and non-asthma patients (17.1% vs 16.6%, unadjusted OR: 1.04, 95% CI: 0.77 to 1.42). In addition, asthma patients were less likely to have severe outcomes (which were defined as death or the need for a high dependency unit or intensive care unit care while hospitalised) than non-asthma patients. Factors associated with asthma management such as pre-admission inhaled corticosteroid use and earlier admission were possible explanations of decreased number of severe outcomes in asthma patients.(103)

The above epidemiological studies have shown a link between influenza infection and asthma exacerbations and other clinical outcomes. Nonetheless, various reasons compromise the interpretation of these studies' findings. First, different diagnostic methods for influenza influence the study outcomes. Second, the use of different definitions and diagnoses for influenza and asthma exacerbations does not allow comparison of findings or provision of pooled robust estimates. Third, inadequate sample sizes and short follow-up periods may mean that given the seasonality of influenza activity a proportion of influenza-induced asthma exacerbations are undetected. Thus, large cohort studies with standardised definitions and diagnoses for influenza and asthma exacerbations including multiple influenza seasons are required to estimate the actual burden of influenza in people with asthma.(104)

Table 1.1: Seasonal and pandemic influenza infection in asthma

Author	Influenza season	Age (y)	Source of cases	Diagnostic method	Respiratory viral, including influenza, induced complications in individuals with asthma
Non-pandemic influenza seasons					
McIntosh[86]	1967-69	1-5	Hospital	Culture; Serum	Respiratory viral infection associated with an exacerbation of wheezing: 58/139 (42%) Influenza associated with wheezing episodes: 1/27 (4%)
Minor[84]	1971-72	3-11	Allergy clinic	Culture	Symptomatic respiratory infection associated with an asthma attack: 42/61 (70%) Influenza associated with an asthma attack: 6/61 (10%)
Minor[85]	1971-72	3-11	O/P private practice	Serum	Influenza associated with a viral respiratory infection: 6/54 (11%) Influenza associated with a severe symptomatic respiratory infection: 3/13 (23%)
Neuzil[87]	1973-93	<15	Database: Medicaid	Culture	Incidence of influenza-associated hospitalisation for acute cardiopulmonary per 1000 children (1 to <3 years): 5.6 Incidence of influenza-associated hospitalisation for acute cardiopulmonary per 1000 children (3 to <15 years): -0.7 Incidence of influenza-associated outpatient visits per 1000 children (1 to <3 years): 190 Incidence of influenza-associated outpatient visits per 1000 children (3 to <15 years): 93 Incidence of influenza-associated antibiotic courses per 1000 children (1 to <3 years): 148 Incidence of influenza-associated antibiotic courses per 1000 children (3 to <15 years): 132
Beasley[94]	1984	15-56	O/P clinic	Culture;	Influenza associated with respiratory illness: 2/23 (9%)

				Serum	Influenza associated with asthma exacerbation: 2/18 (11%) Influenza associated with severe asthma exacerbation: 2/10 (20%)
Johnston[83]	1989-90	9-11	General practice	Serum & Immunofluorescence	Respiratory illness associated with a respiratory tract viral infection: 155/184 (84%) Influenza associated respiratory illness: 21/292 (7.2%)
Nicholson[96]	1990-92	19-46	Multiple	Culture; Serum	Non-bacterial upper respiratory tract infections associated with an asthma exacerbation: 27/61 (44%) Influenza associated with an asthma exacerbation: 1/61 (1.6%)
Teichtahl[95]	1993-94	16-60	Hospital	Culture; Serum	Influenza A and B detected in hospitalised patients due to an asthma attack: 15/79 (19%)
Miller[88]	2000-04	6-59m	Multiple	Culture; RT-PCR	Average annual influenza-attributable hospitalization rates (6-23 months): 2.8 cases per 1000 children Average annual influenza-attributable hospitalization rates (24-59 months): 0.6 cases per 1000 children Outpatient influenza-attributable visits (6-23 months): 316 cases per 1000 children Outpatient influenza-attributable visits (24-59 months): 188 cases per 1000 children
Poehling[89]	2000-04	≤59m	Multiple	Culture; RT-PCR	Out of 160 children with an influenza-related hospitalisation 20 (12%) had asthma Out of 267 children with an outpatient influenza infection 44 (16%) had asthma
Wong[90]	2004-12	<18	Database	Lab. confirmed	Out of 794 children with an influenza-associated death 127 (16%) had asthma

Mandelcwajg[91]	2005-09	1.5 to 15	Hospital; ED	Immunofluorescence	Influenza A detected in hospitalised children for acute asthma exacerbation: 6/232 (2.6%) Influenza A detected in outpatient children for acute asthma exacerbation: 15/107 (14.1%)
Kwon[92]	2010-11	5.3-61m	Ped. hospital	RT-PCR	Prevalence of influenza infection in hospitalised patients with an asthma exacerbation: 7/28 (25%)
2009 pandemic influenza season					
Dawood[102]	2003-09	2-17	Database	Lab. confirmed	During the 2003-09 influenza seasons, 701 (32%) of 2165 children with an influenza-related hospitalisation had asthma During the 2009 H1N1 pandemic, 733 (44%) of 1660 children with an influenza-related hospitalisation had asthma Intensive care due to seasonal influenza: 16% Intensive care due to 2009 pandemic H1N1 influenza: 22% Pneumonia related to seasonal influenza: 40% Pneumonia related to 2009 pandemic H1N1 influenza: 46% Asthma exacerbations due to influenza A: 51% Asthma exacerbations due to influenza B: 29%
Mistry[98]	2008-09	0-19	ED	Multiplex-PCR	Out of 29 children with a 2009 H1N1 pandemic H1N1 influenza infection 9 (31%) had asthma
Salas[99]	2009	7.4-47.4	Hospital	RT-PCR; Rapid test	Out of 46 patients with a H1N1-related hospitalisation 25 (54.4%) had asthma

Jain[100]	2009	21d-86y	Hospital	RT-PCR	Out of 272 hospitalised patients with a 2009 H1N1 pandemic influenza infection 76 (28%) had asthma
Nguyen-Van-Tam[101]	2009	<1 to >75	Hospital	RT-PCR	Out of 631 hospitalised patients with a confirmed A(H1N1)pdm influenza 159 (25%) had asthma
Myles[103]	2009-10	<1 to >86	Hospital	RT-PCR	Oxygen support for patients with a confirmed A(H1N1) influenza hospital admission: 140/385 (36.4%) Pneumonia diagnosis in hospitalised patients with a confirmed influenza A(H1N1): 66/385 (17.1%)
O/P = outpatient; Pulm = pulmonary; RT-PCR = reverse transcriptase chain reaction; ED = emergency department; Ped = paediatric					

1.4 Prevention of influenza infection

Influenza vaccination is the main preventive measure against infection and related complications. However, new vaccines are needed for most years due to antigenic drift the viruses. Thus, WHO makes recommendations on influenza strains that should be included in the vaccine for the forthcoming year. Specifically, WHO has developed the Global Influenza Surveillance and Response System (GISRS) which provides influenza surveillance data which aid the monitoring of influenza activity in different parts of the world.(105) Sentinel practices have been established that receive influenza specimens from people with ILI and submit them to laboratories within national influenza centres. There are 110 such laboratories in 79 countries. When new influenza virus strains emerge, the samples are sent to one of the four WHO reference centres (Atlanta, London, Melbourne and Tokyo) for antigenic analysis. The results from the analysis are then sent to WHO which recommends the strains for inclusion in the new vaccines for the northern and southern hemisphere.(106) WHO in order to increase the efficacy of the influenza vaccines against the most dominant viral strains circulating in the northern and southern hemispheres revises its recommendations on the vaccines' composition twice every year. Therefore, if new dominant influenza strains are circulating in the southern hemisphere, the WHO adjusts accordingly its vaccine composition recommendation for the northern hemisphere and vice versa.(24)(105)

1.4.1 Influenza vaccines

Influenza vaccines can be classified into three broad categories:

- Inactivated vaccines
- Live attenuated vaccines
- Recombinant vaccine

Inactivated vaccines are the oldest (more than 70 years in use) and most commonly used vaccines worldwide. Inactivated vaccines (also called the flu shot) are administrated intramuscular or intradermally. They use an egg-based manufacture technology, where the candidate vaccine strains are grown in hen's eggs for several days until sufficiently replicated and inactivated later before the vaccine is administered.(107) The influenza vaccine with adjuvant is another type of inactivated vaccine (also use egg-based technology), which was approved for the first time in 1997

in Italy. An adjuvant is an ingredient such as the MF-59 which has been added to the influenza vaccine in order to enhance immunogenicity and is usually used in older people (65 years or above) to trigger a stronger immune response.(108)

Cell-based manufacture technology is also used to produce inactivated vaccines, with the first such vaccine (commercially named as "Flucelvax"), approved in November 2012 in USA for adults only (18 years or above). The candidate vaccine strains are produced in a similar way to that of egg-based vaccines, but they are grown in mammalian eggs instead of hen's eggs.(107)

Live attenuated vaccines contain attenuated and cold-adapted influenza viruses generated in temperatures between 25^{o} to 33^{o} (thus unable to replicate at body core temperature) and which have been grown in hen's eggs. It is administered as a nasal spray and it is recommended mostly for young and healthy populations.(107) The immunological benefits of live vaccines compared to the inactivated vaccines include the direct delivery of the viral antigen into the bronchial epithelium through its local administration to nasal passages than parenteral and the wider immunological response.(109)

Recombinant vaccine (commercial name is "Flublok") was approved in January 2013 in the USA. It differs from the previous vaccines as it uses protein-based technology and does not need hen's eggs to grow. The vaccine is produced using only the HA surface protein of the virus, which is known to trigger immune response in humans. It is only recommended for young adults, aged 18 to 49 years.(107)

The current recommended influenza vaccines can further be categorised as trivalent or quadrivalent based on the number of virus strains included in the vaccine. Trivalent vaccines include two subtypes of influenza A virus (e.g., H1N1 and H3N2) and one lineage of the influenza B virus (e.g., Victoria or Yamagata), while quadrivalent vaccines include two subtypes of influenza A virus and both influenza B lineages.(36) Most seasons both lineages of influenza B circulate. Thus, quadrivalent vaccine aims to improve the antigenic match between circulating and vaccine strains and thus offer broader protection against influenza B.(36) Quadrivalent vaccines are also more cost-effective compared to trivalent vaccines. This is because influenza B viruses are more

common in children. Thus, the quadrivalent vaccine provides better direct protection in children and indirect protection in adults by reducing the transmission of the virus from children.(36)

The development of "universal influenza vaccines" with high potency, duration and broad protection is a major priority. This is especially so for low and middle-income countries with limited or no immunisation resources prohibiting the annual seasonal vaccination of whole populations.(110)(111) The universal vaccines can be divided into those aiming to prevent influenza or those aiming to reduce disease severity (e.g. asthma exacerbation).

1.4.2 Milestones of influenza vaccines development

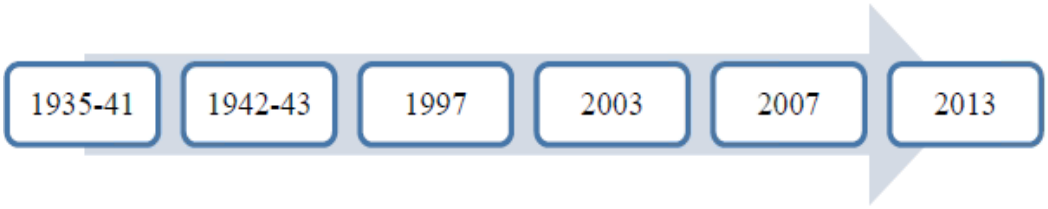

Figure 1.8: Landmarks on the road to influenza vaccines development

In 429 before Christ (BC) the Greek historian Thucydides observed that individuals who survived the smallpox plague in Athens were not re-infected with smallpox.(112) A primitive type of vaccination called "variolation" was first used by the Chinese in 900 anno domino (AD).(112) In 1976, vaccination similar to current forms was discovered by the British physician Dr. Edward Jenner.(112) Jenner used matter from cowpox lesions on the hands and arms of a dairymaid in order to inoculate an 8-year old boy. The boy developed immediately mild symptoms related to infection (e.g. mild fever) who fully recovered after a week. Jenner inoculated again the boy but with matter from smallpox lesions this time. The boy did not present any signs or symptoms related to smallpox which confirmed Jenner's hypothesis that the boy was completely protected. The Latin word for cow and cowpox is *vacca* and *vaccinia* respectively. Thus, Jenner decided to name this new process *vaccination*. Jenner repeated his experiment in a few more cases where the results confirmed his hypothesis. He was therefore the first to prove the protective effects of the vaccination to the global medical scientific community.(113)

In 1931, the American physician Ernest William Goodpasture discovered that several viruses including influenza can be cultivated in the chorioallantoic membrane of chick embryos.(114) Thus, a few years later, in 1935, the first egg-based vaccine was manufactured.(115) Between 1935 and 1941, the first influenza vaccines were tested in humans.(116) During the Second World War (1942-43) influenza vaccines were used for the first time in US army troops in Europe.(117)

In 1946-47 scientists from the US Army Epidemiological Board and from the Office of the Surgeon General observed that strains of the influenza virus can mutate annually.(118) In 1997 the first adjuvant influenza vaccine was licensed in Italy.(81) In 2003, the first live attenuated influenza vaccine was licensed in US.(119) In 2007 the first cell-based technology influenza vaccine commercially named as "Optaflu" was licensed in Europe.(120) In 2013 the first recombinant-based technology influenza vaccine commercially named as "Flublok" was approved by the Food and Drug Administration (FDA) of the US (see Figure 1.8).(107)

1.4.3 Priority groups for influenza vaccination

According to WHO (121) and national committees, some groups should be vaccinated every year due to their high risk of influenza and influenza-related complications. The recommended targets groups include: children 6 months to 5 years old, older people aged 65 and above, pregnant women, persons with certain chronic diseases, health and social care providers, residents in nursing care homes or chronic care facilities and international travellers.(36)(121)

- *Children 6 months to 5 years old* should be vaccinated due to the high risk of influenza and related complications in this age group. Young infants less than 6 months old are not eligible to receive an influenza vaccine and they should be protected through the vaccination that their mother received during pregnancy or through herd immunity provided by its close contacts.(36)(121)
- *Older people* (65 years and above) are recommended for vaccination due to their weak immune system and their high mortality risk.(36)(121)
- *Pregnant women* should be vaccinated with quadrivalent inactivated vaccine (QIV) during pregnancy.(122) The risk of morbidity and mortality due to influenza is substantial in this group. In addition, the morbidity or mortality risk is even

higher in pregnant women with chronic diseases.(36)(121) For example, the risk of hospitalisation in pregnant women with asthma has shown to be 10-fold higher compared to healthy pregnant women.(121) Vaccination during pregnancy also protects young infants (<6 months old). Evidence from randomised trials conducted in low to middle income countries have shown that maternal vaccination reduces the incidence of influenza illness in mothers and their infants.(123-125)Thus, WHO's recommendation on vaccination in pregnancy of any stage protects mothers and their infants under six months.(121)

- *Persons with chronic diseases* such as asthma, chronic heart disease, chronic neurologic disease, chronic kidney disease, chronic liver disease, diabetes, immunosuppression due to disease or treatment, asplenia or dysfunction of the spleen are more likely to infected with influenza.(36) Thus, individuals in this clinically at-risk group should be vaccinated according to immunisation programmes.(121)
- *Residents in nursing homes and patients in chronic care facilities* are highly vulnerable to influenza due the rapid spread of the virus within residencies. Thus, vaccination is crucial in preventing the transmission of the virus and the subsequent influenza outbreak.(121)
- *Persons with morbid obesity* (class III obesity) having a Body Mass Index (BMI) $\geq$40 kg/m^2.(36)

1.5 Rationale for a PhD in influenza vaccines in asthma

I have always been interested in infectious diseases, especially for vaccine-preventable infections which their disease burden can be reduced or even eliminated at a national and global level. As a registered nurse, I have already provided care to patients with infections and I was always recommending and encouraging patients and colleagues to receive all their scheduled vaccines.

During my undergraduate and postgraduate studies, I developed a particular interest in the influenza virus as it is such a genetically unstable virus due to almost annual mutations of its genetic code. Most people think of influenza as a common cold illness that will pass after a few days at home. However, as a healthcare professional I experienced first-hand the severe complications of the virus especially in patients with impaired lung epithelium and antiviral responses such as asthma patients. Thus, I was wondering why people that are at-risk of having serious influenza complications still refuse or undervalue the influenza vaccines, which can be life saving for some of these patients. In addition, why some patients that are immunised against the virus annually still experience complications, given that the vaccines have been in the market for almost 70 years now and many vaccine types have been developed since then.

In 2014, after reading carefully the research proposal for this PhD and the relevant work conducted already by the Seasonal Influenza Vaccine Effectiveness (SIVE) study (126) in Scotland, I realised that the use of a large healthcare administrative dataset is the best method to answer my questions. In addition, this PhD programme was supported by the extensive infrastructure of the Asthma UK Centre for Applied Research (AUKCAR).(127) Specifically, the AUKCAR included a UK-wide postgraduate training platform which provides training on various topics on asthma and on research related skills. Other infrastructure includes programmes related to research methodology and data resources such as the Methodology platform, Asthma Observatory platform and REACH – Database for Research Volunteers. The Patient and Public Involvement platform also supports researchers with their studies by helping them to involve people with asthma during the whole research process.(96) This PhD was also part of the SIVE II project which aimed to assess the effectiveness of the influenza vaccination (as in the SIVE study) but aimed to use a larger scale of

data and subsequently answer more research questions relation to influenza vaccination effectiveness and safety. Working with the SIVE II team also meant that I would be able to get the statistical and epidemiological support needed for this PhD. Any research output would have also been examined by experienced researchers on influenza vaccines which decreases any doubts of the validity of any study findings.(128)

I was unable to find any previous studies investigating the protection providing by the influenza vaccines in the asthma population. I therefore decided that outcomes form this PhD project will be particularly valuable to National Health Services (NHS) in Scotland and other international policymakers to decide whether the seasonal influenza vaccination programme is effective in the asthma population. The findings will also help to maximize the seasonal influenza vaccination programme's impact on the health of the Scottish population with asthma by reducing asthma exacerbations, hospitalisations and deaths that may be viral-triggered.

1.6 Summary to Chapter 1

Respiratory viruses are a common trigger of asthma exacerbations, particularly in children. Influenza virus accounts for only 10-15% of all upper respiratory tract viruses. However, it can cause considerable morbidity and mortality among people living with asthma, as it has been observed during seasons with high influenza infectivity such as in the recent 2009 H1N1 pandemic. The protection against influenza is provided by the seasonal and pandemic vaccines which have been on the market for over 70 years.

Having described the basic concepts of influenza and asthma, the impact of influenza in asthma, the available influenza vaccines and the rationale for this PhD project I will now outline, in the following chapter, the general aim and the individual components of the related empirical studies I undertook for the completion of this programme.

Chapter 2. Aims, objectives and conceptual overview of methods

2.1 Introduction

Preventing influenza among people living with asthma is important because associated illness episodes are generally more severe and more common and vaccination is the main preventive strategy against influenza infection. Thus far, we do not know how many people with asthma take up influenza vaccination, how well influenza vaccination prevents influenza, and how the vaccination prevents the many other associated consequences of influenza among people living with asthma.

2.2 Overall aim

At the beginning of this PhD project, there was only one systematic review, performed by a Cochrane group in 2012 published by Cates et al which assessed the protective effects of the influenza vaccines in asthma. This review used evidence solely from randomised controlled trials (RCTs) up to 2012.(129) By only including RCTs, little evidence was found on the protective effects of the vaccines in people with asthma. In addition, according to the SIVE and AUKCAR researchers there was no up-to-date large observational studies trying to answer this question in the Scottish or UK-wide asthma population despite the UK being among countries with the highest asthma prevalence.(61) The overall aim of this thesis was to estimate the protective effects of the influenza vaccines in asthma. In order to fulfil this aim I carried out three main phases of work including the systematic review of the literature and primary studies which are further described in section 2.4.

2.3 Objectives

The objectives of this PhD research work for people living with asthma were to:

- **Explore** previous literature on the protective effects and safety of influenza vaccines.
- **Measure** vaccine uptake and **explore** the factors influencing the propensity of a person with asthma to receive the vaccine.
- **Estimate** the vaccine effectiveness (VE) in reducing RT-PCR confirmed influenza using a test-negative design (TND) case-control study.
- **Estimate** VE in reducing clinical outcomes including asthma exacerbations, influenza-related primary care consultations, influenza, pneumonia,

respiratory, cardio- and cerebro-vascular hospital admissions and death using a retrospective cohort study design.

2.4 Conceptual overview of the thesis

In this PhD research programme the evaluation of the effectiveness of the influenza vaccine in people with asthma was achieved through successive phases

The **first phase** (Chapter 3) included the identification and assessment of evidence related to the protective effects and the safety of seasonal influenza vaccination in children and adults with asthma through a systematic review. The findings from this phase guided the work undertaken in phases two and four.

The **second phase** (Chapter 5) of the PhD included a TND case-control study in which I assessed the VE against influenza infection using healthcare data over multiple influenza seasons. This enabled the further assessment of the VE and the provision of stratified VE estimates (e.g. influenza season, influenza types and subtypes, vaccine type and age groups).

For the **third phase** (Chapter 6) of my PhD I explored key demographic and clinical characteristics of vaccinated and unvaccinated for influenza people with asthma. I measured the vaccine uptake and conducted a vaccine uptake analysis to explore key characteristics related to vaccination of people with an asthma diagnosis in the community (general practices). The same people with asthma were then followed up in secondary care settings (hospitals) for an additional exploration of the same key characteristics related to vaccine uptake.

The **fourth phase** (it is not yet completed and thus not included in this thesis) involves the assessment of the VE against clinical outcomes (e.g. influenza or pneumonia related primary or secondary medical attendance, influenza-related deaths) in patients with asthma. This phase is currently underway and its completion is estimated after the submission of this thesis.

The synthesis of the thesis was guided from findings from complementary and successive phases of work. Results from phase one which included the review of the literature on vaccination protection and safety were used to inform phase two which included the VE assessment through a case-control study. Phase one will also inform

the ongoing phase four which aims to assess the VE through a retrospective cohort study. Finally, findings from phase three regarding the factors influencing the vaccine uptake will aid the study in phase four to determine which factors need to be included in the final statistical model (see Figure 2.1).

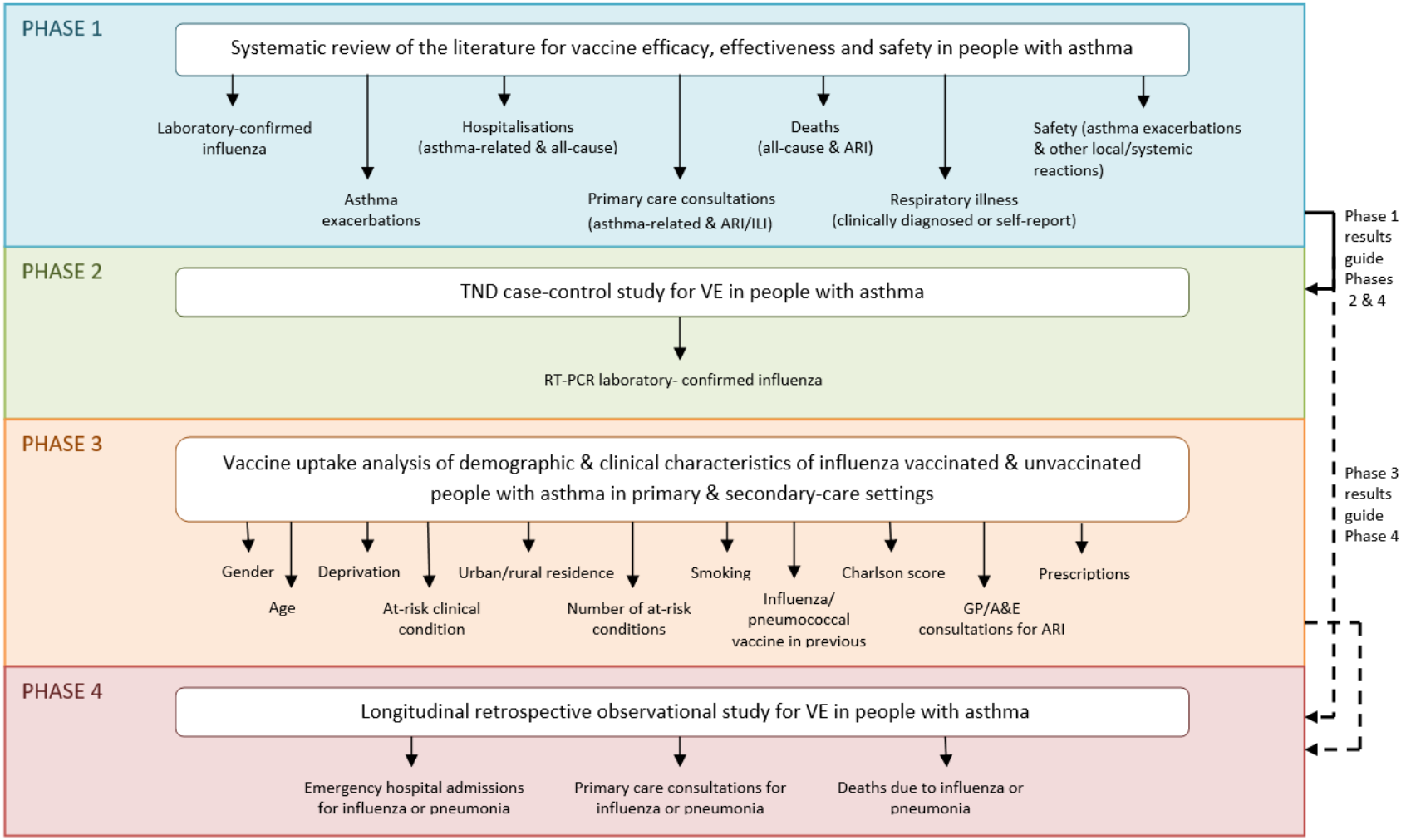

Figure 2.1: Conceptual overview of the PhD

2.5 Evolution of this thesis

The evolution of this research programme was achieved through two main steps (see Figure 2.2):

Systematic review: Before conducting the modelling work to estimate the protection of the vaccines, it was first necessary to interrogate the literature to identify any previous relevant work and gaps. I decided to do this using a systematic review where I investigated the evidence regarding the protective effects and the safety of vaccines in asthma. Searches for published and unpublished literature were carried out independently by two authors (Karim El Ferkh and I) in 2016. All studies found eligible for inclusion by both authors were then appraised regarding quality and any relevant data were extracted. The review showed that the vaccines can protect people with asthma from influenza and accompanying complications, however, the studies supporting this conclusion had several methodological and statistical limitations. Thus, the lack of robust evidence means we still question the protective effects of vaccination in the asthma population. In addition, the safety assessment of the vaccines as a secondary objective of this review could still not answer the evidence gap in live vaccines for young children with asthma (< 6 years old). An evidence gap was also reported in a previous systematic review by Cates et al in 2013.(129) The studies identified in my review guided my primary research work for the provision of robust evidence regarding the protection of vaccines in asthma.

Epidemiological observational studies: The aim was to assess the influenza vaccination programme in Scotland being delivered by primary care to people with asthma using a national longitudinal retrospective observational study. The effectiveness and uptake of the vaccine in asthma was estimated using healthcare administrative data from national laboratory and secondary care databases linked to primary care database provided by the SIVE II project (a 20% sample of the Scottish population).(128) Data covered approximately 20 million patient-influenza seasons from 2000/01 to 2015/16. The effectiveness of the vaccine in preventing laboratory confirmed influenza was assessed using a TND case-control study (see Chapters 4 and 5 for details on this study design). The effectiveness of the vaccine against non-specific clinical outcomes (e.g. asthma exacerbations) was another objective of this PhD

project. However, the results from this work were not available in time for inclusion in the thesis. Unexpected delays related to the access of the healthcare data for this study meant that the data preparation and analyses phases were completed at a later stage. Only preliminary results have been produced by the SIVE II team for the VE against influenza or pneumonia emergency hospital admissions. However, the absence of high-quality evidence (given the presence of bias and confounding related to cohort studies measuring non-specific clinical outcomes – see Chapter 4) for the VE against clinical outcomes means that it was not possible to make any definitive assessments of the VE; hence, additional analyses are required for these outcomes. I also explored characteristics of the asthma population specific to influenza vaccine uptake. Vaccination occurs primarily in the community (general practices). I first explored characteristics of the general asthma population in the community and then followed them up in secondary care to explore characteristics of those that were hospitalised due to influenza or pneumonia (see Chapter 6).

Future work: The evidence gap in safety of the live vaccine in young children with asthma identified during the literature phase of this thesis could not be addressed in this project due to time limitations. However, this research question needs to be answered given that the live vaccine is one of the predominant vaccines administered to children aged 2 years old and above in the UK.

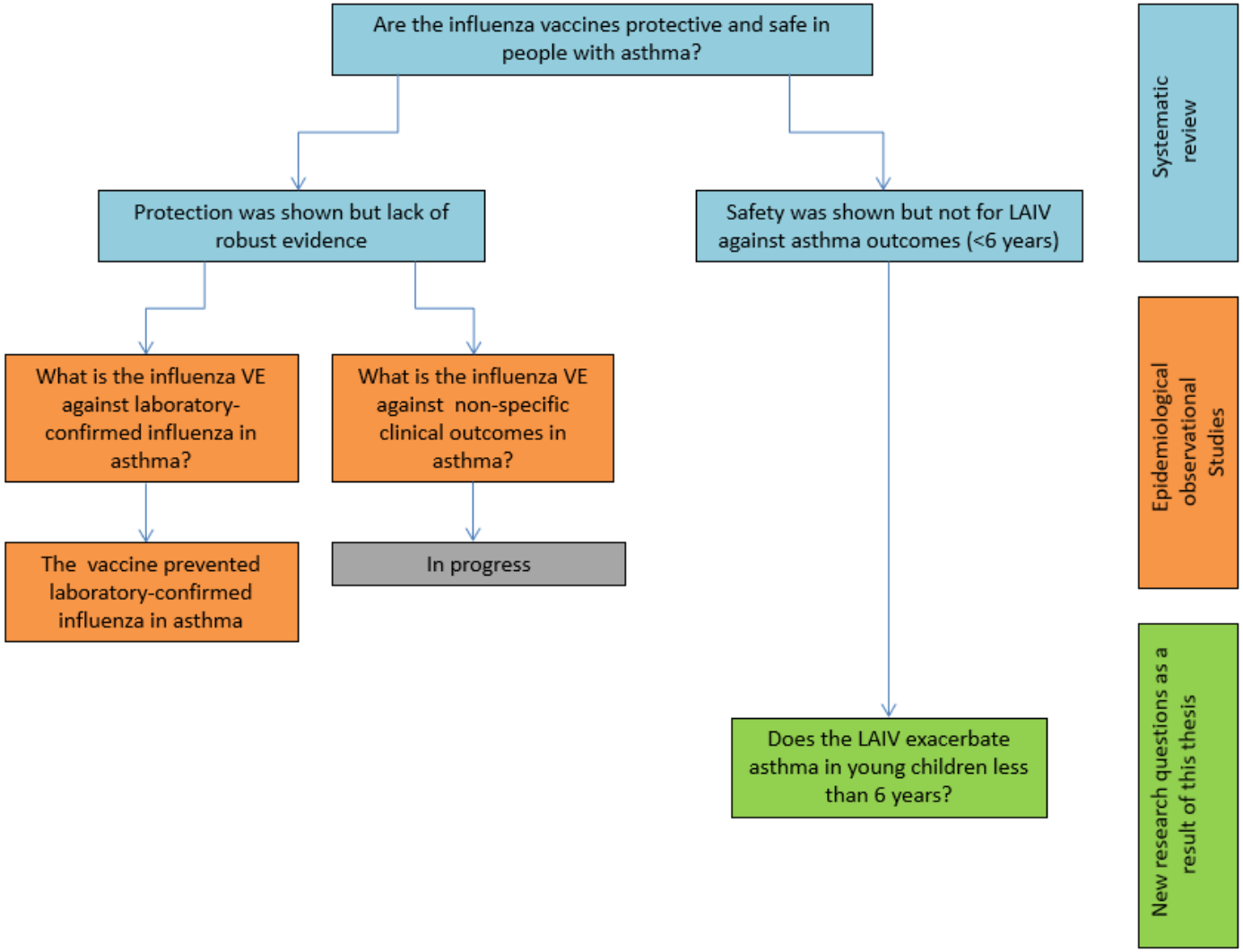

Figure 2.2: Flow diagram illustrating the components of this thesis

2.6 Summary to Chapter 2

In this chapter, I have outlined the aim, objectives, the conceptual overview and the evolution of this thesis. I will now move on to assessing the literature about the effectiveness and safety of influenza vaccines in children and adults with asthma based on clinical and epidemiological studies against laboratory and clinical outcomes.

Chapter 3. Effectiveness and safety of influenza vaccines in asthma: a systematic review and meta-analysis

In this chapter, I will examine the existing published and unpublished literature to identify evidence regarding the protective or adverse effects of influenza vaccines in asthma. The review of the literature including the identification, data extraction and quality appraisal of the eligible studies will be conducted in a systematic and objective approach by two independent reviewers. Relevant qualitative and quantitative synthesis of the literature will also be conducted, if relevant.

3.1 Introduction

Influenza is an acute respiratory illness caused by infection with the influenza virus, which can be severe and, particularly in high-risk groups, result in considerable morbidity and, in some cases, death.(130)(131) Worldwide, influenza causes an estimated five million cases of severe illness and half a million deaths each year. The overall cost to health services in the US has been estimated at US$87 billion per annum.(132)(133)

The burden of influenza is particularly high in individuals with chronic medical conditions.(36) In people with asthma, it is thought that chronic airway inflammation and type-2 immune responses impair antiviral immunity in the respiratory tract,(134) resulting in susceptibility to severe influenza illness and associated bacterial infection. Mechanisms of increased susceptibility to influenza in asthma include weaker innate immune and T-helper 1 cell responses and a deficient interferon alpha response of plasmacytoid dendritic cells to influenza.(74)(134) Furthermore, influenza infections can lead to severe asthma attacks often requiring hospitalisation. In adults, it is estimated that about 20-25% of acute asthma exacerbations leading to emergency department visits or hospital admissions are associated with influenza.(93)

Annual immunisation with influenza vaccine is currently recommended by the WHO and national immunisation technical advisory groups in the US and a number of European and other high-income countries.(36)(50)(121)(135)(136) The uptake in people with at-risk conditions – including asthma – is however well below the target of 75%; it was, for example, only 40% in the US during the 2015-16 influenza

season.(137-139) The reasons for this lack of coverage are complex and multifactorial, but include a lack of confidence in healthcare providers and patients in the effectiveness and safety of influenza vaccine.(140)(141) Decreased perceived risk and severity of influenza may also influence the vaccine uptake.(142) Nonetheless, evidence has shown that about 75% of people with influenza are asymptomatic and 25% of influenza infections are detected by PCR test. Only 17% of people with influenza infection confirmed by PCR test were medically attended.(56) Failure to detect asymptomatic and non-medically attended influenza cases may underestimate the true influenza burden leading to lack of public awareness on the impact of influenza and public misbelief that vaccination is unnecessary.(104) Important in this respect is the hypothesis that the defective mucosal and systemic immunity in asthma (72)(143) may reduce protection provided by influenza vaccines. There may be some grounds to this concern in the context of asthma as a recent Cochrane systematic review (129) investigating the effectiveness of influenza vaccination in those with asthma was inconclusive regarding the efficacy of influenza vaccines. Also of concern is that the safety of live influenza vaccines in infants with wheezing disorders/asthma has not yet been conclusively established.(129) Given that placebo RCTs of influenza vaccination are no longer being undertaken in people with asthma (the last placebo RCT was carried out in 2001 and there is none planned),(144) there is the need to in addition to RCTs also consider evidence from other study designs.(145) We therefore carried out a systematic review and meta-analysis of RCTs and robust quasi-experimental and epidemiological studies to evaluate the efficacy, effectiveness and safety of influenza vaccination in people with asthma.

3.2 Methods

3.2.1 Selection criteria and search strategy

Our methods have been described in detail in our published protocol (146) and our registration of this systematic review in the international prospective register of systematic reviews (PROSPERO) database (CRD42016037219). We included papers which adopted definitions reflecting the heterogeneous definition of asthma.(64) These included people with a history of asthma symptoms and evidence of reversible obstruction of respiratory airways (e.g. bronchodilator reversibility tests or other tests)

in the preceding 12 months, confirmed by a clinician. Patients with asthma were included in the review regardless of their current asthma treatment. Additionally, study participants who had asthma or other chronic medical conditions were included in the review provided that separate data on asthma were available from the study or by personal communication with the authors.

We included studies of influenza vaccines of any type except for pandemic influenza vaccine, any dose and any schedule when compared with placebo, no vaccine or other vaccine. We excluded previous systematic reviews and meta-analyses, which were used only for identifying additional studies.

Our primary outcomes were: 1) a laboratory-confirmed diagnosis of influenza; that is, the incidence or prevalence of laboratory-confirmed influenza infection using any of the available diagnostic tests (RT-PCR or other laboratory tests);(36)(52) and 2) asthma exacerbations.

Our secondary outcomes were: 1) hospitalisations (asthma related and all-cause); 2) deaths (all-cause and respiratory illness); 3) primary care consultations (asthma-related and acute respiratory illness, including ILI); 4) respiratory illnesses (clinically diagnosed or by self-report) and 5) safety, as assessed by asthma exacerbations and other local or systemic reactions.(36)(147)(148)

We included published and unpublished research reports of RCTs or controlled clinical trials and the following observational study designs: TND studies, prospective and retrospective cohort, case-control studies and nested case-control studies.

We searched the published literature from January 1970 to January 2016 for studies investigating the effectiveness of influenza vaccination in people with asthma. This start date was chosen because the evidence on this subject began to accrue following publication of the paper by Bell *et al* (149) in 1978.(150)(151)

We searched MEDLINE (Ovid), EMBASE (Ovid), Cumulative Index to Nursing and Allied Health Literature (CINAHL), Cochrane Central Register of Controlled Trials (CENTRAL), Scopus, Cochrane Database of Systematic Reviews (CDSR), Web of Science Core Collection, Science direct, WHO Library Information System (WHOLIS), Global Health Library and Chinese databases (CNKI, Wanfang and ChongQing VIP) for published experimental and observational studies. Electronic searches were complemented by manually searching reference lists of included papers for additional studies. In addition, forward citation search was performed on all identified studies using Web of Science. Furthermore, unpublished or ongoing clinical trials were searched in clinical trials registry databases using http://www.controlled-trials.com/, http://www.clinicalTrials.gov/ and the WHO International Clinical Trials Registry Platform (ICTRP) using http://www.who.int/ictrp/en. We contacted pharmaceutical companies that manufacture influenza vaccines used in the trials to identify additional published or unpublished studies. Authors of the studies included in the review were contacted when we required additional information. There was no language restriction. Specific search strategies were developed for each database (see appendix A1; p 239).

3.2.2 Risk of bias assessment and data extraction

Two reviewers (EV and KF) independently assessed the risk of bias, and disagreements were resolved through discussion or by the involvement of a third reviewer (CS). The risk of bias of experimental studies was assessed using the criteria outlined in the Cochrane Collaboration's tool (152) according to the following six domains: 1) sequence generation; 2) allocation concealment; 3) blinding of participants and personnel and outcome assessors; 4) outcome ascertainment; 5) selective outcome reporting; and 6) other bias. Risk of bias in included studies was divided into three categories: high, low, or unclear. The overall risk of bias rating was based on the suggested algorithm in the Cochrane Collaboration's tool.(152) Specifically, overall low risk of bias was assigned to a study with low risk of bias ratings across all six domains, overall unclear risk of bias to a study with unclear risk of bias ratings in one or more domains and overall high risk of bias to a study with high risk of bias ratings in one or more domains.

The Quality Assessment Tool for Quantitative Studies Dictionary developed by the Effective Public Health Practice Project (EPHPP) was used for the evaluation of observational studies and non-randomised controlled studies.(153) The EPHPP tool contains the following eight components: selection bias (external validity), study design (allocation bias), confounders, blinding (detection bias), data collection methods, withdrawals and dropouts (attrition bias), intervention integrity and analyses. The first six components are rated as strong, moderate, or weak. There was no rating for the last two components. The overall study rating was judged as strong, moderate, or weak based on the component ratings. Specifically, the overall quality of a study was assigned as strong in the absence of weak ratings across all six components, moderate in the presence of one weak rating and weak in the presence of one or more weak ratings. Relevant data from studies eligible for inclusion were extracted by two reviewers (EV and KF), independently, using a data abstraction form designed for this review (see appendix A2; p 251). Any disagreement upon the extraction of data of the included studies was resolved through discussion or by the involvement of a third reviewer (CS).

3.2.3 Grading the quality of evidence

The strength of evidence for each outcome was assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) Working Group methodology.(154) This evaluation was based on the following five domains: risk of bias, consistency, directness, precision and publication bias. There were four possible levels of quality: high, moderate, low, and very low. The GRADEpro software was used to determine the overall quality rating for each outcome. However, the GRADE tables were adjusted based on components that are more suitable for the current review.

3.2.4 Data analysis

Separate meta-analyses were performed for clinically and methodologically comparable experimental and observational studies in order to estimate the incidence or frequency of influenza infection (laboratory confirmed) and febrile illness. Random-effects models were used to summarise the findings depending on the degree of clinical heterogeneity of the studies. For dichotomous outcomes, the treatment

effect was estimated using a risk ratio (RR) with 95% CI or OR with 95% CIs. Vaccine efficacy and VE is usually reported as a percentage e.g. (1-OR)*100. Safety data from cross-over trials could not be pooled together due to lack of adequate data regarding the two cross-over periods. Statistical heterogeneity was assessed using the standard χ^2 test and the I^2 statistic, which describes the proportion of dispersion across studies due to true heterogeneity rather than to a sampling error (0-100% heterogeneity). We contacted authors of included studies that had missing data. All statistical analyses were undertaken using RStudio version 0.99.893.(155)

We were unable to perform any sensitivity, subgroup analyses due to small number of studies included in our meta-analyses. Similarly, publication bias assessment could not be conducted as the number of studies included in each meta-analysis was too small.

3.3 Results

3.3.1 Selection of studies and study characteristics

Our initial research identified 20,396 unduplicated records. After screening of titles and abstracts, 318 potentially eligible studies were selected for full review. Thirty-two studies eligible for inclusion were identified through database searches and a further three studies through reference screening. We therefore included 35 studies enrolling 142,519 patients with asthma in qualitative synthesis and four studies in the meta-analyses (see Figure 3.1).(144)(149)(156-188)

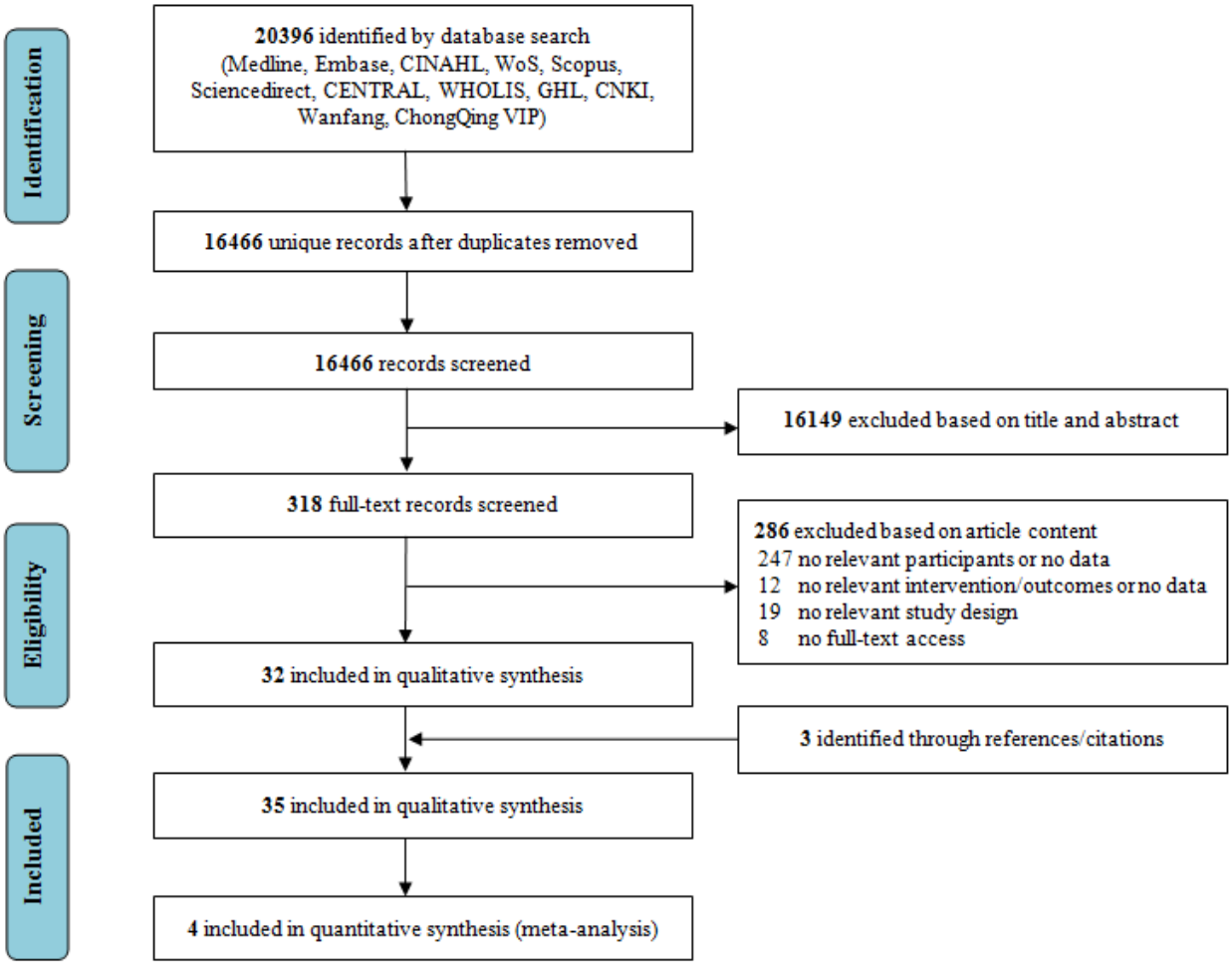

Figure 3.1: PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) diagram

Fifteen studies were conducted in Europe,(144)(157)(159)(161-163)(166)(167)(170-172)(174)(177)(186)(187) 10 in the US,(149)(156)(158)(164)(169)(180)(182-185) eight in Asia,(160)(165)(173)(176)(178)(179)(181)(188) one in Mexico,(168) and one in Australia.(175) Data for people with asthma were provided by two (184)(185) out

of 35 studies. A third study identified from references (161) did not report data separately for people with asthma. However, *Cates et al* (129) were able to obtain these data and we therefore included the data reported by *Cates et al* in our review. No eligible studies were found through trial registries or influenza vaccine manufacturers. Twenty RCTs were included,(144)(149)(156-173) six non-RCTs,(174-179) seven cohort studies (180-183)(186-188)and two case-control studies were also identified.(184)(185)

Seventeen studies recruited children as study participants.(144)(149)(157)(159)(160)(165)(168)(169)(173)(176)(179-183)(186)(187) Ten studies included adult participants (156)(161)(163)(164)(166)(170)(171)(174)(177)(178)and seven studies included both children and adults.(160)(162)(172)(175)(184)(185)(188)No age range of patients was reported in one study.(167) Inactivated influenza vaccine was the intervention in 22 studies,(144)(149)(157)(158)(161-164)(166-168)(170-172)(174-179)(187)and live attenuated vaccine (LAIV) in four studies.(156)(165)(169)(173) One study compared live with inactivated vaccination.(159) However, eight studies did not specify the type of vaccination.(160)(180)(182-186)(188)

The protective effects of the vaccine were assessed in 20 studies against our primary and/or secondary outcomes of interest. Specifically, laboratory confirmed influenza was assessed in seven studies (159)(165)(170)(173)(184-186) (three RCTs, one non-RCT, one cohort study, and two TND case-control studies), asthma exacerbation in seven studies (144)(160)(174)(179)(181)(182)(188)(two RCTs, two non-RCTs, three cohort studies), hospital admissions in six studies (149)(160)(174)(179-181)(two RCTs, two non-RCTs, two cohort studies), primary care consultations in two studies (159)(180)(one RCT and one cohort study), respiratory illness in eight studies (144)(164)(173)(174)(179)(181)(186)(187)(three RCTs, two non-RCTs, three cohort studies), emergency department visits in three studies (160)(180)(181)(one RCT and two cohort studies), increased use of asthma medication in two studies (160)(181) (one RCT and one cohort study), pulmonary function in one study (143) (one non-RCT) and school or work absenteeism in one study (159) (one RCT). Safety of the vaccine

against asthma attacks, local and systemic adverse reactions, healthcare use, influenza infection, respiratory illness, pulmonary function, asthma medication and work or school absenteeism was evaluated in 19 RCTs,(149)(156-173) four non-RCTs,(175-178) and one cohort study.(183)

3.3.2 Risk of bias assessment in individual studies

We preferentially looked at data from RCTs. The overall risk of bias was high in five RCTs, unclear in 12 RCTs and low in three RCTs. The overall quality of 12 studies (six non-RCTs and six cohort studies) was rated as "weak". In two case-control studies and in one cohort study, the overall quality was rated as "moderate" (see Figures 3.2 & 3.3 and Appendices; A3-A6; p 266-276).

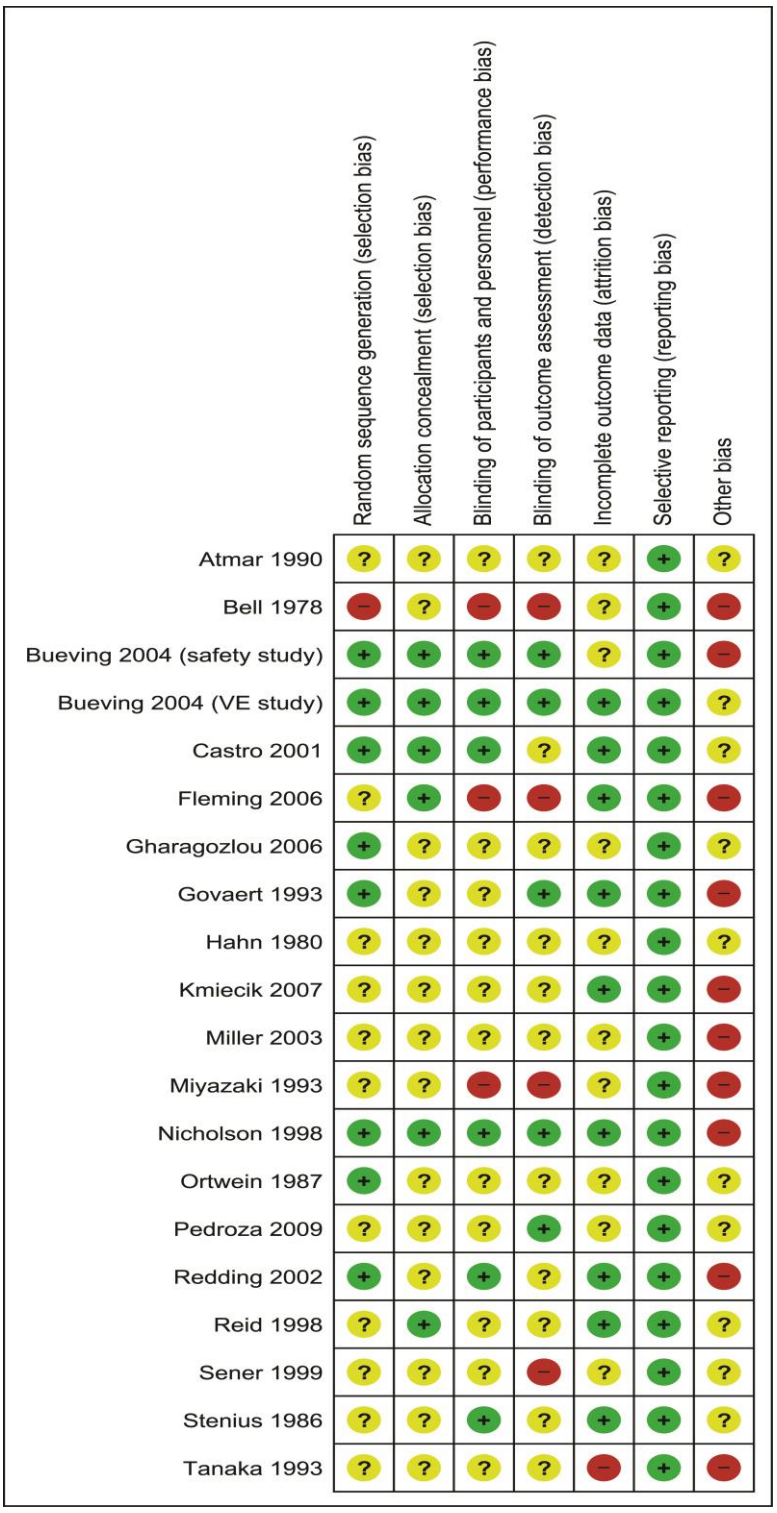

Figure 3.2: Risk of bias summary: review authors' judgments about each risk of bias item for each RCT. This rating was based on the Cochrane guideline

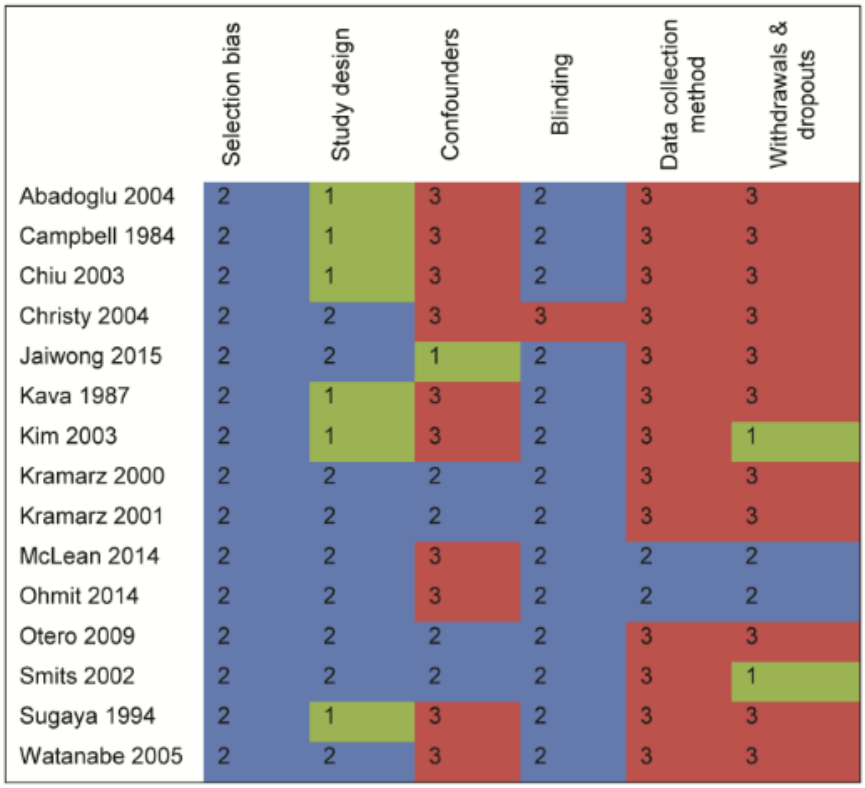

Figure 3.3: Quality assessment of the non-RCTs and observational studies using the EPHPP quality assessment tool

3.3.3 Overall quality of evidence

The body of evidence regarding influenza VE and safety regarding primary and secondary outcomes was rated using the GRADE approach as being of very low quality due to inconsistency, indirectness, and imprecision across studies. In addition, the strength of evidence for the protective effects of vaccination against pulmonary function and school or work absenteeism was rated as very low since the evidence was based on single studies. Thus, the consistency, directness, and precision of the pooled overall estimation could not be assessed. Similarly, the evidence of safety of influenza vaccination against influenza infection and respiratory tract illness was assigned as very low as it was provided by single studies (see appendix A7; p 277).

3.3.4 Vaccine efficacy and effectiveness against influenza infection

Nosocomial outbreaks of A (H1N1) and B subtypes were observed during two consecutive years (1988-89 and 1989-90) among 84 children with asthma.(165)(173) Protection provided by LAIV in these children against laboratory confirmed influenza was found in two small RCTs (pooled VE 81%; 95% CI: 33 to 94; see Figure 3.4). A large multicenter RCT evaluated the efficacy of the live vaccine compared to the inactivated vaccine against community-acquired culture-confirmed influenza illness in children (aged 6-17 years).(159) The efficacy of LAIV was significantly higher than the inactivated influenza vaccine. The live vaccine efficacy against influenza subtypes antigenically similar to those included in the vaccine was 35% (95% CI: 4 to 56).

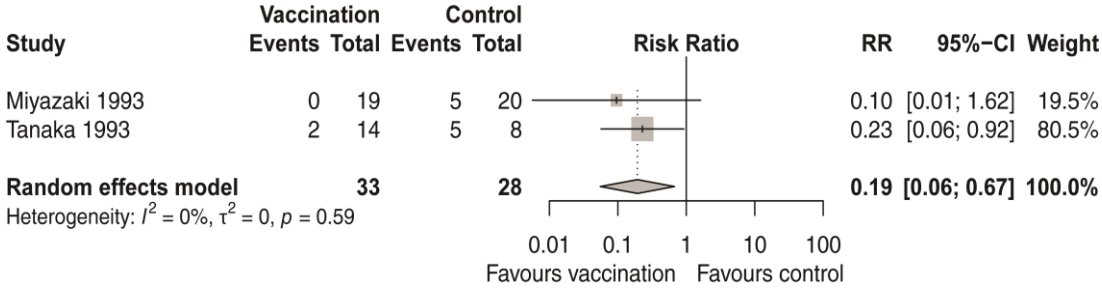

Figure 3.4: Live attenuated influenza vaccine versus no vaccine against influenza infection (RCTs)

A meta-analysis was undertaken of two TND studies performed in the US during the seasons 2011-13.(184)(185) In 2011/12, the influenza vaccine was well matched and influenza A H3N2 predominated with A H1N1 and both influenza B (Victoria and Yamagata) also circulating.(189) In 2012/13, H3N2 again predominated in the US with a late season predominance of influenza B.(190) The influenza VE for people with asthma ranged was 38% (95% CIs -0.05 to 63·0) in 2011/12 and 46% (95% CIs 32 to 58) in 2012/13. Once these results were pooled, we found an overall VE of 45% (95% CI 31 to 56; see Figure 3.5) in preventing influenza (RT-PCR laboratory confirmed) in 1,825 individuals with asthma (aged ≥6 months).(184)(185)

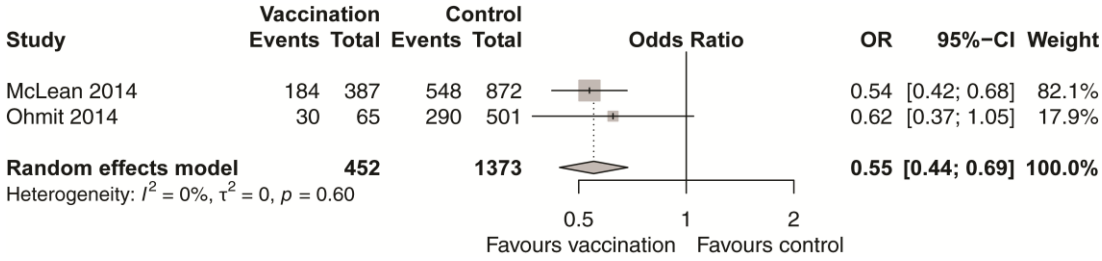

Figure 3.5: Seasonal influenza vaccine versus no vaccine against laboratory confirmed (RT-PCR) influenza infection (test-negative design studies)

One prospective cohort study assessed the effectiveness of the influenza vaccine in preventing influenza in 338 children during the 2005-2006 season. There were no laboratory confirmed influenza infection cases in the vaccinated group, while eight (4·4%) unvaccinated children had an infection.(186) In a non-RCT, the overall efficacy of inactivated vaccine was 42% (95% CIs 21 to 57) against influenza infection (diagnosed by virus isolation or HI antibody titre increase) in 137 children (aged 2-14 years).(179) During the epidemic period, antigenic drift was observed in A(H3N2) flu strain. However, the efficacy against the A (H3N2) subtype was higher (VE 68%) than of the well-matched B subtype (VE 44%), particularly in children $\geq$7 years old ($p<0.01$).

3.3.5 Vaccine efficacy and effectiveness against asthma attacks and other clinical outcomes

The protective effects of vaccination against asthma exacerbation were also observed in four studies.(160)(181)(182)(188) One RCT (160) found protective effects of the influenza vaccine against the incidence, frequency and duration of asthma attacks in 201 children (aged 1-15 years). Acute asthma attacks were lower in the vaccinated group (39/79) compared to the unvaccinated group (82/122) (RR 0.73; 95% CI: 0.57 to 0.95).

VE against asthma attacks was also studied in three observational cohort studies.(181)(182)(188)In the first study, inactivated influenza vaccine provided higher protection against asthma attacks (defined as wheezing episodes) (mean $\pm$ S.D.: 1·6 $\pm$ 1.6) compared to the unimmunised group (mean $\pm$ S.D.: 6·2 $\pm$ 3.9) ($p<0.001$).(181) The second study provided evidence regarding reduction in attacks after controlling for asthma severity and other confounders. Protective incidence rate

ratios were observed for the 1994-5 season (0·59; 95% CI: 0·43 to 0·81), and the 1995-6 season (0·65; 95% CI: 0·52 to 0·80), but not for the 1993-4 season (0·78; 95% CI: 0·55 to 1·10).(151) In the third study, the rate of asthma attacks was significantly (p=0.037) lower in the vaccine group (mean ± S.D.: 0·14 ± 0·4) compared to control group (mean ± S.D.: 0·35 ± 0·61) during the 2002-3 season, but not in the 2001-2 season.(188)

Six studies assessed VE in preventing hospitalisations due to asthma attacks or respiratory infections (e.g., pneumonia, influenza-like illness and pharyngitis).(149)(160)(174)(179-181)A RCT assessed the duration of hospitalisation for ILI accompanied by asthma, ILI and asthma alone in 93 children (aged 6-16). The length of hospitalisation for ILI alone ($p<0·01$) and ILI accompanied by asthma ($p<0·05$) was significantly lower in the bivalent inactivated vaccine group compared to the unvaccinated group.(149) In a cohort study, the number of hospitalisations was 0·2 ± 0·6 (mean ± S.D.) among the inactivated vaccine recipients and 1·3 ± 1·5 (mean ± S.D.) among controls ($p<0.001$).(181)

Two studies (159)(180)assessed the protective effects of vaccination against asthma or respiratory illness consultations. A retrospective cohort study reported higher visits to a paediatric clinic among vaccine recipients (2.14) than in the unvaccinated ones (0.71; OR: 2·9; 95% CI: 2·0 to 4·1).(180)

VE against respiratory illness was found in four studies.(179)(181)(186)(187) Pooled estimates regarding live attenuated VE against febrile illness were estimated from two RCTs.(165)(173)Pooled VE of 72% (95% CI: 20 to 90; see Figure 3.6) was observed against febrile illness during two nosocomial outbreaks with A (H1N1) and B subtypes.(165)(173) In another trial, clinical efficacy of inactivated subunit vaccine against febrile influenza illness was 49% (95% CI: 24 to 66) in 137 children (aged 2-14 years) ($p<0.01$). A higher vaccine efficacy (74%) was observed in children at least seven years old ($p<0.01$).(179)

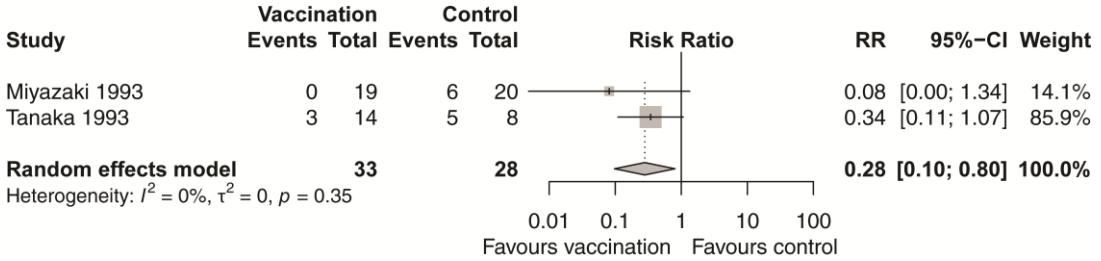

Figure 3.6: Live attenuated influenza vaccine versus no vaccine against febrile illness (RCTs)

The protective effects of vaccination against respiratory illness were also reported in three cohort studies. In the first study, the number of respiratory tract illnesses were significantly lower (mean ± S.D.: 2·2 ± 2·1) in the inactivated vaccine recipients compared to the unvaccinated group (mean ± S.D.: 6·9 ± 3·9) (p<0.001).(181) The second study found that 0·6% of vaccine recipients had a RSV infection compared to 2·5% of controls. In addition, protective effects of the vaccine were also observed against other respiratory infections (RR: 0·61; 95% CI: 0·29 to 0·95) and bronchiolitis (RR: 0·47; 95% CI: 0·26 to 0·84).(186) In the last study, the effectiveness of the inactivated subunit vaccine was 56% (95% CI: 18 to 76) against acute respiratory disease (defined as ILI, bronchitis, bronchiolitis, asthma exacerbation or otitis media) during the 1996-7 season. In particular, higher VE of 77% (95% CI: 35 to 92) was found in younger children < six years old.(187)

The VE in preventing asthma-related emergency department (ED) visits was evaluated in three studies.(160)(180)(181)A cohort study observed lower ED visits for asthma exacerbations among inactivated vaccine recipients (mean ± S.D.: 0·4 ± 0·9) than the unvaccinated group (mean ± S.D.: 2·2 ± 2·6) (p<0.001).(150) In contrast, another cohort study of vaccinated children had more ED visits for asthma or pneumonia (OR 2·0; 95% CI: 1·2 to 3·1).(180)

The protective effects against increased use of asthma medication were also reported in two studies.(160)(181) In a RCT, the frequency of bronchodilators use was lower in the vaccinated group (35/79) compared to unvaccinated group (77/122; OR 0.46; 95% CI 0.26 to 0.83).(160) A cohort study reported significantly (p<0.001) higher number of bronchodilator administrations in the unvaccinated group (mean ± S.D.: 6·2 ± 3·9) than the inactivated vaccine group (mean ± S.D.: 1·6 ± 1·6). Similarly,

prednisolone administrations were significantly ($p<0.001$) higher in the unvaccinated group (mean ± S.D.: 1·1 + 1·2) compared to vaccinated group (mean ± S.D.: 0·1 ± 0·3).(181) No improvements in pulmonary function and reduction in work/school absenteeism were found from influenza vaccine.(159)(174)

3.3.6 Safety of influenza vaccines

The safety of the vaccination against asthma exacerbation was measured in eight studies.(158)(159)(163)(166)(169)(169)(178)(183) One cross-over RCT (166) reported moderate to severe exacerbation of asthma within 72 hours following immunisation with inactivated vaccines vs. placebo. The risk of asthma exacerbation was considerably higher in the first-time vaccine recipients (1 in 16) compared to second-time vaccine recipients (1 in 83). However, the increased risk of exacerbation was no longer significant after excluding patients with colds or incomplete symptom diaries. A cohort study found higher risk of influenza-vaccine asthma exacerbations among the vaccine recipients. However, the incidence rate ratios were 0·58 (95% CI: 0·36 to 0·95), 0·74 (95% CI: 0·47 to 1·17), and 0·98 (95% CI: 0·76 to 1·27) after controlling for severity of asthma and other potential confounders during all three seasons (1993-96).(183)

The risk of local and systemic adverse reactions, including asthma-related symptoms following vaccination was estimated in 20 trials.(157-163)(165-173)(175-178)However, significant differences were observed in eight trials.(157-160)(166)(167)(170)(173)Side-effects (fever, body pain, sore throat, cough, rhinorrhoea, headache, and malaise) were reported in nine (11·4%) out of the seventy nine vaccine recipients and none in the placebo group within two weeks after immunisation.(160) The number of systemic symptoms and all symptoms were significantly higher in the inactivated vaccine group than the placebo within three days following vaccination.(166) Erythema (23%) and painful or stiff arm (48%) were more frequent among inactivated vaccine recipients than placebo during the first week after vaccination.(157) Influenza-like illness symptoms including fever (8%), headache (10%), myalgia (18%), and hoarseness (5%) were also more often reported in the vaccine group.(144) Cough (8%) was the only asthma symptom that was found to be significantly higher in the vaccine group than placebo.(157)

Myalgia was significantly higher in the inactivated vaccine group (25·1%) than placebo group (20·8%) two weeks after vaccination.(158) One month after immunisation with inactivated vaccine eight patients developed systemic symptoms (sore throat, fever, and malaise) and one patient developed a local reaction at the injection site. No adverse reactions were found in the placebo group.(170) Redness at the site of injection was the only local adverse effect within six weeks following immunisation with inactivated subunit, split and placebo injection.(167) Diarrhoea was the only significant systemic adverse reaction in five patients after the first vaccination (mainly after the split vaccine), and in four patients after the second vaccination with the subunit vaccine.(167)

The safety of LAIVs against local and systemic adverse reactions was also assessed in four RCTs.(159)(165)(169)(173)Rhinorrhoea was significantly higher in live vaccine recipients (80%) than placebo controls (52%) within one week following immunisation.(173) The incidence of adverse events was similar between the live and inactivated vaccine groups within 15 and 28 days after immunisation. However, LAIV recipients reported runny nose or nasal congestion (66·2% Vs 52·5%), rhinitis (7·4% Vs 3·9%) and headache (6·5% Vs 4·2%) more often than TIV recipients while wheeze was observed more often in the inactivated group (23·8%) than live vaccine group (19·5%). In addition, local reactions at the injection site were reported in 70·8% of TIV recipients. 59·8% of TIV recipients reported pain at the injection site.(159)

Pulmonary function deterioration following vaccination was assessed in 16 clinical trials.(149)(156)(158)(159)(162)(166-172)(175-178)Three studies found a decrease in lung function after immunisation.(149)(166)(175) However, the decrease in lung function was not accompanied with an exacerbation of asthma (asthma symptoms, increased medication or health-care use). No significant differences were reported post-vaccination for use of asthma medication, health-care use, influenza, respiratory illness, and work or school absence.(156-158)(166)(178) We found four non-RCTs (175-178) and one observational study (183) (not included in the Cates review [129]).

These papers found that the influenza vaccine led to no increase in post-vaccine asthma attack or symptoms when compared to placebo (for non-RCT studies) or no vaccine (observational studies) (see Figure 3.7).

	Asthma exacerbation	Asthma symptoms	Adverse reactions	Health-care use	Asthma medication	Influenza infection	Respiratory illness	Pulmonary function	School/work absenteeism
RCTs									
Atmar 1990				No	No	Yes	Yes	No	
Bell 1978					Yes			Yes	
Bueving 2004		Yes	Yes	No	No				No
Castro 2001	No	No	Yes	No	No				No
Fleming 2006	No	No	Yes					No	
Gharagozlou 2006			Yes						
Govaert 1993			No						
Hahn 1980			No					No	
Kmiecik 2007	No		Yes						
Miller 2003	No								
Miyazaki 1993			No						
Nicholson 1998	Yes		Yes	No	No			No	
Ortwein 1987			Yes					No	
Pedroza 2009			No					No	
Redding 2002	No	No	No		No			No	
Reid 1998		No	Yes		No			No	
Sener 1999		No	No		No			No	
Stenius 1986		No			No			No	
Tanaka 1993			Yes						
Non-RCTs									
Campbell 1984		No			No			No	
Chiu 2003		No						No	
Kava 1987			No		No			No	
Kim 2003	No	No		No	No			No	
Observational study									
Kramarz 2000*	No								

Figure 3.7: Safety outcomes in experimental and observational studies
*Asthma exacerbations were significantly higher only in the 1993-94 influenza season (p-value: 0.03).

Summary of all studies' findings are also provided in appendix (see Appendix A8; p 279). Additional graphical presentation of the VE against primary and secondary outcomes is provided in appendix (see Appendix A9; p 292).

3.4 Discussion

Our findings indicate that there is some evidence supporting the effectiveness of influenza vaccination to prevent influenza and other clinically important health outcomes in people with asthma. In particular, pooled estimates from observational TND studies suggest that influenza vaccination is beneficial against laboratory-confirmed influenza (VEs ranging from 38-46%, with a pooled estimate of 45%).(184)(185) The effectiveness of vaccination in reducing asthma exacerbations, healthcare use, respiratory illness and medications for asthma was also identified.(149)(160)(165)(173)(179)(181)(182)(186)(188) However, much of this evidence comes from observational studies and therefore bias, residual confounding and confounding by indication are alternative possible explanations. Also, for each outcome the quality of the body of evidence (across all included studies) was very low according to the GRADE methodology.

There are several reasons why there is a need to consider evidence from robust quasi-experimental and observational studies. A Cochrane review of RCTs on this subject, which found inconclusive evidence to support influenza vaccination in those with asthma,(129) whilst well conducted, was however of limited value to decision makers, clinicians or patients. This is because there have been no relevant placebo RCTs over the last 15 years and no new trials are in progress or planned as it has been considered unethical to withhold vaccination, particularly from those most at risk of severe influenza illness. There are various reasons why RCTs of influenza vaccination in people with asthma are not conducted. First, influenza vaccines are included in public health guidelines, therefore RCTs are automatically disallowed as there is no uncertainty regarding the benefits of the vaccines in asthma patients. Second, new influenza vaccines are developed with the assumption that they are more efficacious than current vaccines. Third, RCTs will delay providing any results in a timely manner given that the influenza strains change constantly from season to season or even within the season. For example, it would be unethical to conduct an RCT for the new vaccines and delay giving life-saving immunisation protection to at-risk populations. Finally, even a large and robust RCT will only be able to provide subject, place, time and influenza strain and vaccine strain specific results. This is because influenza vaccine strains change every season, new influenza vaccines are developed every few years,

circulating influenza strains change by time (e.g. season) and the subjects' pre-existing immunity from previous exposure to influenza (or the influenza vaccine) varies by time, place and age. Therefore, ethical and logistic reasons would render RCTs inappropriate and improper for estimating the protective effects in current and new influenza vaccines in asthma patients.(191)(192)

Furthermore, observational TND studies are now being used to help inform national advisory bodies on their influenza vaccination programmes. For instance the Advisory Committee on Immunization Practices (ACIP) has not recommended the use of LAIV in the US during the 2016/17 seasons.(193) Evidence of no effectiveness (3%) of LAIV vs. good effectiveness of inactivated vaccine (63%) from US based TND studies was the primary reason for this decision.(193) In 2018/19 season ACIP started recommending the LAIV for children aged 2 to 17 years old.(194) Evidence from the UK LAIV programme shows VE particularly for the influenza B and A(H1N1) strains. Specifically, in 2016/17 season the overall VE was 66% while no overall significant VE was observed in 2017/18 season. However, in 2017/18 season high VE of 60% and 90% was found for the influenza B and A(H1N1) strains.(195)(196) Therefore, the findings from the UK on the LAIV VE justifies the continuation of the LAIVs in children. In addition, amongst children with a history of asthma or wheezing asthma superior efficacy of LAIV was found compared to trivalent inactivated influenza vaccine.(197) Therefore, further research to establish the effectiveness of LAIV amongst children with asthma using observational study data is required.(128)

Most studies differed by recruitment methods, vaccine ascertainment methods, type of vaccines and outcome definitions (in some cases outcomes were not described) (see appendix A10; p 294). Particularly, the definition and evaluation of asthma exacerbations is an important point of variability across studies (see appendix A10; p 294). Most studies (experimental and observational) also recruited children or adults less than 65 years old. Thus, only a few studies have assessed the effectiveness of influenza vaccination in older people with asthma.

Influenza infection was confirmed by viral cultures or by a $\geq$ 4-fold rise of antibody titre, in three RCTs.(165)(173)(179) However, the low sensitivity of these tests might

affect the VE estimates. Residual immunity from previous vaccination may have also affected VE estimates in two studies.(184)(185) Similarly, residual immunity from previous seasons might have also underestimated the VE in another study.(187)

In a RCT (159) about 70% of patients were on steroid therapy and 31% had a history of asthma hospitalization. Thus, asthma severity could have decreased the vaccine efficacy. Similarly, VE was found in a cohort study (182) only after adjustment for asthma severity. No VE against health-care use was found in another cohort study.(180) However, if only children with severe asthma were more likely to be vaccinated that could explain the lack of VE amongst the vaccinated group.

With the small number of studies included in each meta-analysis, publication bias could not be adequately assessed. Our planned subgroup or sensitivity analysis for VE could not be carried out due to lack of data from the included studies.(146) In particular, evidence is needed for VE against influenza B and influenza A sub-types. Also, more in-depth analyses, which include the number, nature and antigenic distance specified by virus mutations across sequential circulating variants and vaccine components and the role of prior vaccination are required.(198) This will require larger TND studies with pooling of data across regions and countries. No new substantive evidence for LAIV safety, beyond the vaccine safety studies included in the Cates' review,(129) was found.

Public health initiatives are required to improve the current low vaccine uptake in people with asthma.(139) Evidence from clinical trials and observational studies suggests likely benefits for people with asthma of vaccination against influenza infection, respiratory illness, asthma attacks and other influenza-related asthma complications including asthma related emergency department visits and hospitalisations.

3.5 Summary to chapter 3

The literature indicates that influenza vaccines provide some protection against laboratory and clinical complications and they do not cause any severe adverse events in asthma individuals. However, the evidence regarding the VE estimates derived mostly from observational studies with inherited methodological and statistical

limitations. The methodological limitations when estimating the influenza VE in observational studies will be further discussed in the following chapter before the provision of any VE estimates using observational studies as aimed.

Chapter 4. Estimation of influenza vaccine effectiveness in observational studies

This chapter aims to define the terms vaccine efficacy and effectiveness which are commonly used in clinical trials and observational studies, respectively. The distinction between the two terms is crucial when assessing the protective effects of the influenza vaccines in order to understand what aspects of the vaccine are assessed during an epidemiological study. The factors that influence the measurement of the protection provided by the influenza vaccines in observational studies are also described in this chapter.

4.1 Definitions

During the development of a new influenza vaccine, many pre-licensure trials are conducted to assess its efficacy to prevent a potential influenza infection. Vaccine efficacy reflects only the potency of the vaccine itself to protect against influenza. Its measurement is quantified by comparing the reduction of influenza infection between vaccinated and unvaccinated groups. Vaccine efficacy is ideally measured through a RCTs, where the ideal and controlled conditions of a trial permit its unbiased estimation.(199) Specifically, the following three conditions exist when efficacy of the vaccine is measured in a RCT:

1) Both vaccinated and unvaccinated groups should have the same exposure rate to influenza virus.
2) The exposure to influenza vaccination should be randomly allocated among study participants
3) Vaccine efficacy estimates the direct benefit of vaccination in vaccinated compared to unvaccinated - the unit of the study is the individual, and not the whole population.(50)

During the post-licensure period the potency of the vaccine at a population level needs also to be assessed. A clinical trial can assess the vaccine potency at population level. For example, a large clinical trial can follow-up a population over multiple consecutive influenza seasons to estimate the VE. Alternatively, a multi-centre clinical trial can be designed to assess the VE during one influenza season across various geographical

locations at national or international level.(159) The design of the clinical trials would therefore overcome any sample size issues allowing the real-time VE population.

However certain challenges arise when VE is estimated in clinical trials such as the required follow-up period and the type of measured outcome . For example, VE against a laboratory-confirmed influenza infection outcome should be assessed during a whole influenza season (e.g. September in year 1 until end of influenza circulation in year 2). Every influenza season is unpredictable in terms of the volume and type of circulating influenza strains therefore the follow-up period for this outcome should be at least one influenza season. This time length allows a sufficient number of influenza cases to be included in the trial to empower any planned analyses.(200) Nonetheless, the long follow-up observation period will increase the cost and the operational complexity of the clinical trials. In contrast, the assessment of the immune response induced by the vaccines via serologic tests requires a shorter follow-up time period (e.g. 1-3 months) but not less than two weeks since full immune protection is achieved 14 days post vaccination. This study endpoint measures the rise in antibody titers as a result of body's exposure to influenza virus via exposure to vaccination. However, a problem with serology testing is the existence of antibodies from previous influenza infection or vaccination. Pre-existing antibody against influenza will cross-react with antibodies generated from current vaccination. Therefore, the interpretation of the observed rise in antibody titers is challenging. Some studies overcome this uncertainty by comparing the rise in antibody levels between pre- and post-vaccination blood samples.(200)

Thus, the assessment of VE via epidemiological observational studies is a more appropriate measurement to monitor how well the vaccine can protect against influenza in relation to vaccine programs and population-related characteristics.(201) VE refers to the reduction of influenza infection and other influenza-related complications in vaccinated individuals compared to unvaccinated in a real-life setting rather than in an ideal environment such as a controlled clinical trial. VE is usually measured by observational studies in a population or sample population.(50)

Both vaccine efficacy and effectiveness refer to the percent reduction in incidence among vaccinated individuals due to influenza vaccination and can be estimated using the following formula:

$$VE = \frac{IRU - IRV}{IRU} = 1 - RR$$

The reduction of incidence rate among unvaccinated (IRU) is subtracted by the incidence rate among vaccinated (IRV) individuals and then divided by the IRU. The VE formula is also equal to one minus the RR. In studies that the RR cannot be estimated the OR can be used instead. Thus, VE will also be equal to 1-OR.(50)

4.2 Factors influencing the estimation of influenza vaccine effectiveness in observational studies

The estimation of influenza vaccine effectiveness is influenced by many factors, which can be grouped under the following five broad categories: study design, vaccination exposure, population, influenza virus and its match or mismatch with vaccine strains, and confounding. The consideration of all these factors is crucial during the design and conduct of an observational study for the provision of unbiased VE estimates (see Figure 4.1).(50)

Study design

Type of outcomes

Most observational studies include specific clinical outcomes (laboratory confirmed influenza), non-specific clinical outcomes (ILI, acute respiratory infection, hospitalisation, death due to any cause) or a combination of the two.

Individuals at high risk for influenza are more likely to be hospitalised due to influenza or other related consequences. Thus, in observational studies the outcome of hospitalisation is usually attributed to various reasons such as influenza, pneumonia, cardiovascular or other causes. However, these are not specific outcomes and may influence the true VE estimates.(202)

Death due to influenza infection is rarely recorded on medical records. The complication of influenza infection is usually recorded as the death cause and not the infection-trigger. For example, pneumonia is a common complication of influenza and it is usually recorded as the primary cause of death. Thus, researchers use various causes of death, which may not specifically be related to influenza.(202)(203)

Outcomes such as acute respiratory infection and ILI are usually diagnosed through clinical signs and symptoms. However, other non-influenza respiratory viruses can induce similar clinical symptomatology to influenza. Additionally, the definition of ILI or acute respiratory infection based on signs and symptoms differs among countries or does not exist. The symptomatology of ILI is also affected by age. For example, the signs and symptoms of ILI in older people may not reflect the severity of the illness. Specifically, older people may have serious infection, which may not be accompanied by serious signs and symptoms.(204-206)

Laboratory confirmed influenza is considered the most precise outcome for the estimation of VE. Influenza infection is diagnosed through the real-time RT-PCR laboratory technique, which has become the gold standard for detection of influenza virus due to its high sensitivity and specificity.(204-206)

Influenza vaccines provide protection only against influenza viruses and not other respiratory viral pathogens. In addition, influenza illness has similar signs and symptoms with other respiratory viral illnesses. Thus, an ILI cannot be attributed solely to influenza virus. The specificity of the ILI also varies by the activity of the influenza and other respiratory viruses. For example, in seasons with high influenza activity ILI may be more specific as a higher proportion of the illness will attributed to influenza compared to other co-circulating respiratory viral pathogens. However, the specificity of the ILI would still be lower compared to a laboratory confirmed influenza infection. As a result, the use of non-specific clinical outcomes (e.g. ILI) underestimates the true VE in a population. Clinical outcomes based on laboratory confirmed influenza should be used to assess VE in a population.(207)

Population source for cohort studies

Administrative databases, sentinel general practice networks and institutions are some of the common sources to obtain a target population for a cohort study. Specifically, databases such as population registries, immunisation registries, primary care records and insurance scheme databases can be linked using a unique identifier for each individual in some countries. The linkage of various databases provides information including general practice records, hospital records, death records and vaccine records.(50)(208)

Some issues using administrative databases include external validity. The sample included in the database should be representative of the source population. For example, some individuals may have lower access to health care and may not be included in the database. Thus, the external validity of the database will be lower due to lack of representativeness of the general population.(50) In addition, the accuracy of health records is another issue to be considered. Specifically, accuracy refers to agreement between the information provided in databases to those in health records. The agreement between medical records and computer databases varies according to previous studies in the UK and in the USA. However, all studies reported high accuracy level of data recorded in databases.(209-211) Completeness of the computer databases should also be considered. For example, the completeness of coded morbidity data in 41 Scottish practices was about 75%.(211)

Sentinel GP networks are another population source for cohort studies that exist in most European Union (EU) countries. These networks are part of the surveillance scheme in each country. They detect cases with ILI and for a sample of patients presenting with influenza like symptoms confirm the infection using swabs which are sent to virology laboratories (according to the surveillance regulations).(209)(212)

Institutions such as long-term residencies or care facilities are another source of populations for a cohort study. However, issues including the generalisation of the target population and the difficulty of finding unexposed individuals due to the high vaccine coverage rate need to be taken into account.(213)(214)

Population source for case-control studies

Cases can be obtained from general practices, hospitals, nursing homes and death registries. However, the selection of controls from each of the above sources should be carefully determined and several issues should be considered.(50)

Specifically, controls for cases from general practices can derive from a random sample. For example, cases may be patients with laboratory confirmed influenza or with ILI and controls could be a random sample of patients from the GP practice in question (which in turn may include some of the cases).(50) Additionally, cases may be patients that were diagnosed with influenza - those with a negative diagnosis can be used as controls. Specifically, general practitioners (GPs) are taking swabs from patients with ILI symptoms. Patients' samples are then tested for influenza through the real-time RT-PCR laboratory technique. This is a new type of case-control study, referred to as a TND, where patients with a positive laboratory test result are characterised as cases and those with a negative test result serve as controls. The estimation of VE through test-negative design is considered to be more precise due to laboratory confirmed flu using the real time RT-PCR test. Additionally, the selection of controls from ILI cases (with a negative test result) reduces the methodological problems and costs of a case-control study. Thus, TND as a new type of case-control studies has been proved to be of high validity.(50) Finally, cases can be patients with ILI from sentinel general practices, while controls may be obtained from health surveys that provide information on influenza vaccination of interviewees.(50)

Hospitals are another source of cases. Cases are usually patients hospitalised for pneumonia and influenza or cardiovascular and respiratory diseases (and in some cases from people admitted for any cause). Controls can be a random sample from the hospital, general practice or community.(50) In addition, cases can be derived from a long-term care or resident facility and controls could be those without the outcomes of interest. However, VE may be overestimated when the incidence of influenza is high, and control participants' vaccine coverage may be less representative of the nursing home as a whole.(50) Death registries can also be used to obtain cases, whereas

controls can be derived from the community, a database or from the influenza season following the season where cases were obtained.(50)

Cases can be easily selected from various sources. However, the selection of controls should be always representative of the source population. Specifically, the vaccine status of controls should represent that of the source population. Thus, randomly selected controls are more likely to have a vaccine coverage representative of the source population and VE is less likely to be over- or underestimated.(50)(215)

Vaccination exposure

Vaccine status ascertainment is usually based on medical records, immunisation records, health insurance or through interviews. Thus, the vaccination status of the study individuals can be confirmed by more than one source.(215)

The time between the date of vaccination and the development of antibody titres should be taken into account, when the vaccine status is determined. Some studies consider an individual vaccinated 14 days following the vaccine administration, while other studies consider an individual vaccinated the day of vaccination. However, the immune system requires approximately 14 days to develop sufficient immune memory following the exposure to vaccine strains.(209) Finally, some studies have shown that vaccination in previous seasons may influence the protection provided by the vaccine in the following season. Thus, vaccination history should be taken into account when assessing VE.(216)

Population

In older people, the vaccine may be less effective due to immune senescence and therefore there is a lower immune response to vaccine strains.(217) Similarly, in very young individuals (6 months to 3 years old) the influenza vaccine may be less effective due to their immature immune system or the absence of immune memory.(218) Furthermore, VE in individuals with immunosuppression (e.g. COPD, HIV-infected patients) may be lower due to their vulnerable immune system.(219)(220) VE in people with severe asthma (e.g. Step 5 of the British Thoracic Society Guidelines) may

be lower due to modifying effects caused by oral corticosteroids.(221) The impact of immunosuppressants (e.g. oral or inhaled corticosteroids) on vaccine protection in individuals with respiratory conditions (e.g. asthma) has also been assessed. No significant differences were reported between individuals on medium-dose or high-dose inhaled corticosteroids or oral corticosteroids compared to individuals on low-dose inhaled corticosteroids or not on corticosteroids.(221) However, more studies are required to assess any modifying effect of steroids and other immunosuppressant medication in individuals with respiratory conditions due to paucity of evidence as identified in a systematic review.(222)

In addition, individuals with co-morbidities and old age are more likely to be vaccinated as one of the target populations for annual vaccination.(223) The source of population may also affect the VE. For example, individuals in long term residencies or care facilities are more likely to be vaccinated.(224)

Influenza virus

VE is influenced according to the activity of the influenza virus each season. Specifically, VE may be underestimated in small studies during seasons with low influenza incidence.(225)

The similarity (match) between the circulating influenza virus and the influenza strains included in the vaccine is also important. For example, in seasons where there is a good match between the virus and the vaccine strains, VE is more likely to be effective.(226)(227)

Confounding

Confounding by indication and healthy vaccine effect are the common confounders found in observational studies measuring VE. "*Confounding by indication is a term used when a variable is a risk factor for a disease among nonexposed persons and is associated with the exposure of interest in the population from which the cases derive, without being an intermediate step in the causal pathway between the exposure and the disease".*(228)

Confounding by indication occurs when individuals at high risk for influenza infection are more likely to be vaccinated compared to those with lower risk. Thus, VE may appear lower because more people at high-risk for influenza are vaccinated.(215) Due to a lack of randomisation of the vaccination allocation in observational studies, confounders such as comorbidities should be taken into account when measuring VE. The healthy vaccine effect occurs when healthy individuals are more likely to be vaccinated compared to those with higher risk for influenza and it may be less likely that individuals with decreased functional status (i.e. those near to death) are vaccinated.(50) Thus, VE against non-specific clinical outcomes such as pneumonia, heart and respiratory disease, hospitalisation and death may be overestimated.(215)

4.3 Summary to chapter 4

The efficacy of vaccines refers to the ability of a vaccine to prevent an infection in an ideal setting and thus is usually estimated in clinical trials, while the effectiveness of vaccines refers to the protective ability of a vaccine in real time settings and at a population level. Since the VE is estimated at a population level through observational studies, various factors can potentially hamper the provision of true estimates that cannot be fully controlled due to the nature of the study design.

In this chapter, I provided the definition of VE and explained how the most common factors influencing its estimation particularly in observational epidemiological studies. The theoretical knowledge acquired in this chapter will enhance the understanding of the next chapter which involves a test-negative design case-control observational study. Therefore, the methodology of the study in terms of how the VE was measured and what are its main strengths and limitations in comparison to other common observational studies measuring VE will be now more easily understood.

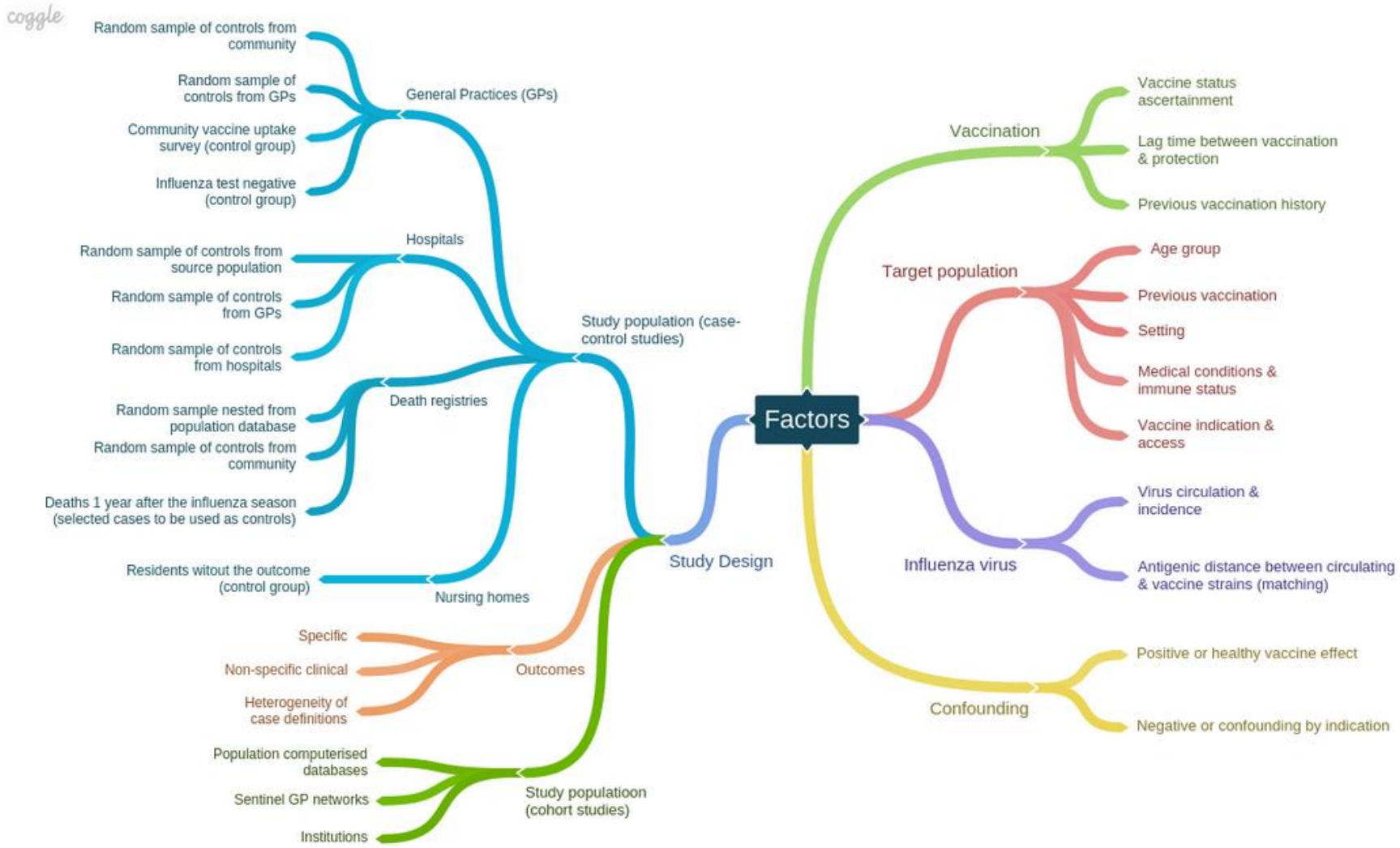

Figure 4.1: Factors influencing VE estimation in observational studies

Chapter 5. Seasonal influenza vaccine effectiveness for the prevention of laboratory-confirmed influenza in a Scottish asthma population: a test-negative design case-control study

The studies reviewed in Chapter 3 have shown the protective effect of the influenza vaccination against influenza infection in asthma. The low study quality combined with the inadequate sample size in those studies made it difficult to give a robust answer regarding the vaccine protection. Thus, in Chapter 4 I discussed how VE is measured in observational studies and methodological issues that need to be considered in these studies. In this chapter, I therefore aim to assess the preventive ability of the influenza vaccines against influenza infection using a more methodologically and statistically robust study with an adequate sample. As a result, this study will enable the answer of the main and any other secondary questions leading to a full exploration of the benefits of the vaccine in the asthma population.

5.1 Introduction

The majority of national immunisation committees assess the effectiveness of the influenza vaccines based on evidence from observational studies rather than placebo RCTs which are no longer conducted in people with asthma (the reasons are discussed in the section 3.1 of Chapter 3 describing the systematic review).(145) Specifically, the VE for each influenza season is determined based on the TND case-control study, which is becoming the gold standard for generating precise VE estimates.(229-231) The TND study evaluates the influenza VE on patients that seek care for acute respiratory infection. Cases are those testing positive for influenza and controls are those testing negative for influenza. In essence, the TND involves identifying those with proven influenza infection and comparing the proportions of those who were vaccinated with those who were unvaccinated. VE is calculated by (1 - OR)*100%, where OR is the ratio of the odds of being vaccinated in cases versus the odds being vaccinated in controls.(231) According to Jackson et al "*This study avoids confounding by care-seeking, as the study population is restricted to those who would seek care if they developed an ARI"*.(230) As a result, TNDs studies are less prone to selection bias that may happen in case of a strong positive relationship between vaccine receipt and health-care seeking behaviour which may increase the possibility of subject recruitment.(231) Thus, any differences in health care-seeking behaviour between

vaccinated and unvaccinated individuals are reduced in a TND study. However, studies with specific (influenza related) end-points that are restricted to single seasons may be underpowered to provide a reliable VE estimate.(145)(232) Another advantage of the TND study is that it can be cheap and produce rapid results since these studies can be nested in routine national surveillance systems.(231) The speed of the TND study also allows the provision of interim within-season VE estimates which can be used as a proxy for end of season VE estimates and guide health professionals on influenza prevention.(233)

The majority of the TND studies do not set out to explicitly assess the VE in individuals with asthma, but in those with chronic respiratory conditions including asthma.(234) As a result, only two TNDs were identified in the systematic review.(234) Two case-control studies published after the search date of my literature review assessed VE in individuals with asthma based on more than one influenza season.(235)(236) Specifically, the study by Suearez-Varela et al compared the VE between asthma and non-asthma hospitalised patients for laboratory-confirmed influenza.(235) However, the VE in asthma was inconclusive which was expected given that less than 20% of cases and controls had asthma and included only patients aged 65 years or above during two seasons.(235) In the second study, the authors assessed the VE in children 6-59 months during four seasons by various characteristics including asthma.(236) VE in the asthma subgroup was significant and up to 43.3% (95% CI: 17.1 to 61.2). However, no further analyses in relation to other demographics or other characteristics related to influenza infection or the vaccination were performed for the asthma subgroup.(236)

Based on evidence from the systematic review in this thesis and subsequently published, relevant studies, VE in asthma remains understudied. Specifically, various characteristics related to the asthma population, influenza infection, and the different vaccine types are still to be explored.

The aim of this study was to assess the VE in children and adults with asthma. More specifically, the objectives of this study were to: (1) evaluate the seasonal influenza VE across and in single seasons; (2) evaluate the VE against common seasonal circulating viral strains; (3) provide VE estimates for by age groups; (4) assess the VE

between live and inactivated vaccines in children; (5) assess the VE in a combination of previous and current vaccination history and (6) assess the VE by location of swab collection (primary versus secondary care).

5.2 Methods

The SIVE II study was an observational study which aimed to evaluate the effectiveness and safety of the seasonal live and inactivated influenza vaccines in children and at-risk groups respectively.(128) Therefore, the assessment of the VE in people with asthma was part of the objectives of the SIVE II study. The SIVE II study included linked routinely administrative healthcare data sources from primary care, secondary care and laboratory datasets in Scotland.(128)

I was part of the SIVE II team as evidenced by my significant intellectual contribution to the development and writing of both the published study protocol (128) and the final study report to the National Institute for Health Research – Health Technology Assessment. I conducted the TND case-control study against laboratory-confirmed influenza in people with asthma which was guided by the SIVE II study.

I also led additional analyses within the TND case-control study which were asthma specific and neither pre-specified in the published protocol of the SIVE II study nor included in the final report of the SIVE II study. I provided VE estimates for all pre-pandemic (before the 2009/10 pandemic) and post-pandemic seasons for asthma while the final report of the SIVE II study included only VE estimates on post-pandemic seasons. In addition, I conducted a number of subgroup analyses which were not specified in the SIVE II published protocol. Specifically, these analyses included VE estimation by age group, vaccine type, vaccine history, predominant influenza A subtypes and primary or secondary care settings of swab collection.

5.2.1 Study design and population

I used a TND case-control study to estimate VE against laboratory-confirmed influenza in children and adults with asthma by pooling data from 14 influenza seasons (2000/01 to 2015/16). Various influenza A(H1N1) subtypes circulated predominantly during seven of the seasons (2000/01, 2007/08, 2010/11, 2012-14, 2015/16). However, the influenza A/California/07/2009 strain of the A(H1N1) subtype was observed in the

seasons following the 2009 A(H1N1) pandemic. The A(H3N2) influenza A subtype dominated in nine seasons (2001-05, 2006/07, 2008/09, 2011-13, 2014/15). Influenza B co-dominated during nine seasons (2000/01, 2002/03, 2004-06, 2008/09, 2010/12, 2012/13, 2014-16) with other influenza A subtypes (see Chapter 1 for details on these strains). Only during the season 2012/13 all influenza A and B types/sub-types co-dominated – this was characterised as a mixed season.

The cohort of people with asthma was defined as ever general practice diagnosis of asthma using Read codes.(237)

Patients were swabbed in either general practices (sentinel and non-sentinel) or hospitals and tested for influenza as they had symptoms compatible with influenza. The multiplex RT-PCR assay - a laboratory test with the highest rapid and predictive detection rates for acute respiratory tract infections - was used to determine influenza positivity.(238) Patients with a positive test for influenza were classified as cases, while those with a negative test for influenza were classified as controls (see Figure 5.1). A person could not contribute more than one swab per season. For negative swabs the first was used and for patients with a positive and negative swab the first positive was used. In patients with more than one positive swab only the first positive swab was used. It is possible that the same patient appeared in more than one seasons but this is very rare. VE estimates were yielded by comparing the influenza vaccine uptake between cases and controls.(239)

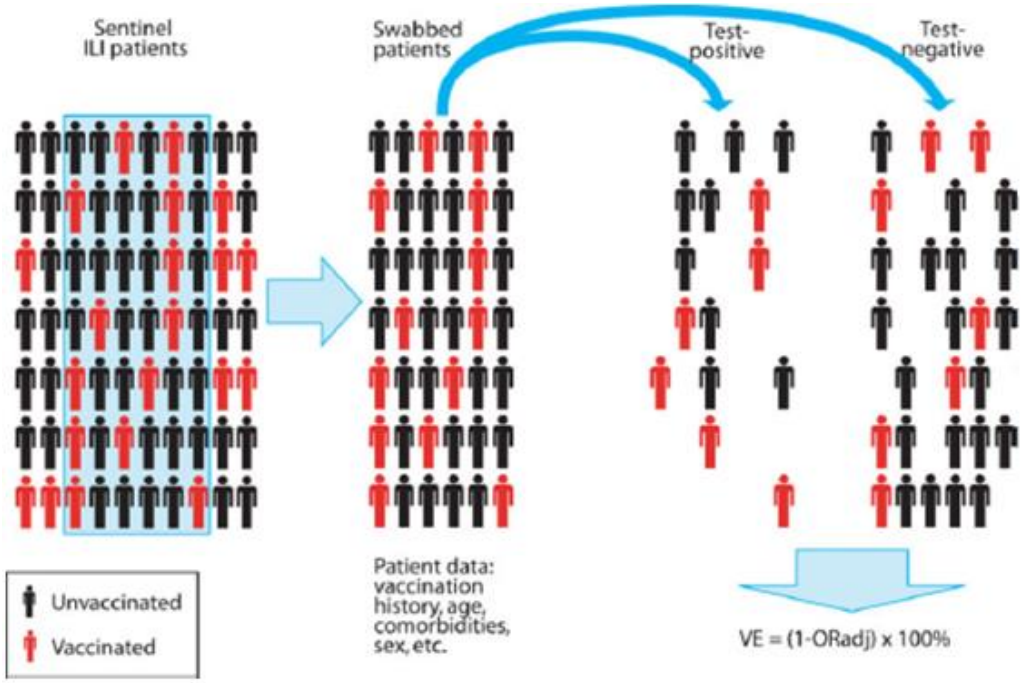

Figure 5.1: Test-negative design case control study for influenza VE estimation based on swab samples taken only from sentinel surveillance settings
Reproduced with permissions from: Sullivan SG, et al. Potential of the test-negative design for measuring influenza vaccine effectiveness: a systematic review. Expert Rev Vaccines 2014; 13(12): 1571-91.

5.2.2 Study databases

Virtually the entire Scottish population has access to primary healthcare that is free at the point of delivery and is registered with a primary care practice. Each resident in Scotland has a Community Health index (CHI) number, which is a unique patient identifier adopted by the NHS. The linkage of the following databases was carried out using the CHI number.(128) The electronic Data Research and Innovation service (eDRIS) as part of the National Services Scotland (NSS) carried out the linkage of the databases, hosted the analytical dataset and provided a trusted research environment for the statistical analysis.(128)

Primary care: GPs provide primary care services to almost all residents in Scotland and hold information about vaccine administration (including information on pharmacy immunisation where passed on the GP by the pharmacist) and medical history data.(128) Additionally, they provide services such as prescriptions and decide who needs referral to a hospital or other health specialist facility. They facilitate the transition of a patient from a hospital back to the community by supervising and

providing care when required.(128) Data provided by Scottish general practices are considered of high quality and their valuable contribution to epidemiological research has been previously proven.(211)(240) Specifically, the Information Services Division (ISD) of NHS Scotland has reported that the completeness and accuracy of coding included in general practice data are over 91%.(240) Annual influenza vaccinations are performed mostly in primary care practices and in less extent in pharmacies (except for the live attenuated vaccine which is administered in primary schools from 2013 in Scotland).

Sentinel GP-based swabbing scheme: GPs are also part of the sentinel swabbing scheme which is currently set up in 100 general practices across all 14 NHS Health Boards in Scotland. Each participating GP has to take up to five nasal/throat swab samples obtained from GP consultations with patients with influenza-related symptoms on a weekly basis. Thus, the first five people with ILI symptoms presented in a sentinel GP are swabbed which could mainly refer to an opportunistic sampling method as some people may also refuse to be swabbed. Then, sentinel GPs send these swab samples to the Scottish Influenza Surveillance Reporting Scheme (SISRS) based at Health Protection Scotland (HPS), which monitors the activity of the influenza virus every season in Scotland. In this study swab samples were also collected from non-sentinel GPs and secondary healthcare settings (for diagnostic purposes) which exceeded the number of swab samples derived from the sentinel GPs for surveillance purposes. The SISRS is also using a number of other complementary surveillance systems (e.g. NHS24 cold/flu calls, severe influenza monitoring or influenza vaccine uptake and antiviral prescribing patterns) in order to assess the activity of the virus and the success of the control measures (e.g. vaccination) since a single surveillance system cannot provide all the required information.(241)

Scottish Immunisation & Recall System: The Scottish Immunisation & Recall System (SIRS) is a database that holds records of all vaccinations including the influenza vaccine administrated in children less than six years according to the UK childhood immunisation schedule.(242) Thus, data on influenza vaccines administrated predominantly in schools were obtained through SIRS. The

administration of seasonal inactivated and live influenza vaccines was determined via the use of Read codes.(237)

Electronic Communication of Surveillance in Scotland: The Electronic Communication of Surveillance in Scotland (ECOSS) division of the HPS is a database that holds records on various microorganisms (e.g. influenza virus) and infections reported from participating diagnostic laboratories.(243) However, in this study only data on RT-PCR tests for influenza were accessed from the ECOSS database. Patient identifiable information is also collected, which enabled linkage with vaccination records (see Figure 5.2). Data on all laboratory tests carried out in non-sentinel primary and secondary health care facilities were also collected by the ECOSS database.

5.2.3 Recruitment process and characteristics of the GPs in this study

Scottish primary care practices were invited to assist the SIVE II study through a formal electronic invitation. An information sheet was also designed to provide more detailed information about the research project. The SIVE II study recruited 223 GPs through the Scottish School of Primary Care (SSPC) in collaboration with Albasoft Ltd.(244) The SSPC facilitated collaboration between academics with significant primary care research output and key stakeholders in primary care. Albasoft Ltd was the trusted third party that carried out the data extraction from all 223 GPs using the Enhanced Services Contract Reporting Options (ESCRO) system. ESCRO is an email/web based system that supports GPs by providing enhanced services (e.g. payment system for GPs) within NHS Scotland.(245) ESCRO also provides healthcare informatics services to Scottish Universities such as provision of data extraction facilities.(245) Thus, GPs were not actively involved in the data extraction (other than permissions as data custodians). The data extraction was carried outside of working hours of the GPs to avoid any interference with their daily operations. The SIVE II study also reimbursed each participating general practice with a £50 administration fee. Out of the 223 GPs, 40 were also part of the of the sentinel surveillance system for influenza in Scotland.(241) These sentinel GPs are encouraged (through financial incentives) to take swabs from up to ten patients with influenza illness each day. The recruitment of the 223 GPs was broadly representative of the Scottish primary care population by gender, age and socioeconomic status. However, no additional specific

information are known for these 223 GPs since only anonymised data where retrieved and no GP identifiers were available in this study.

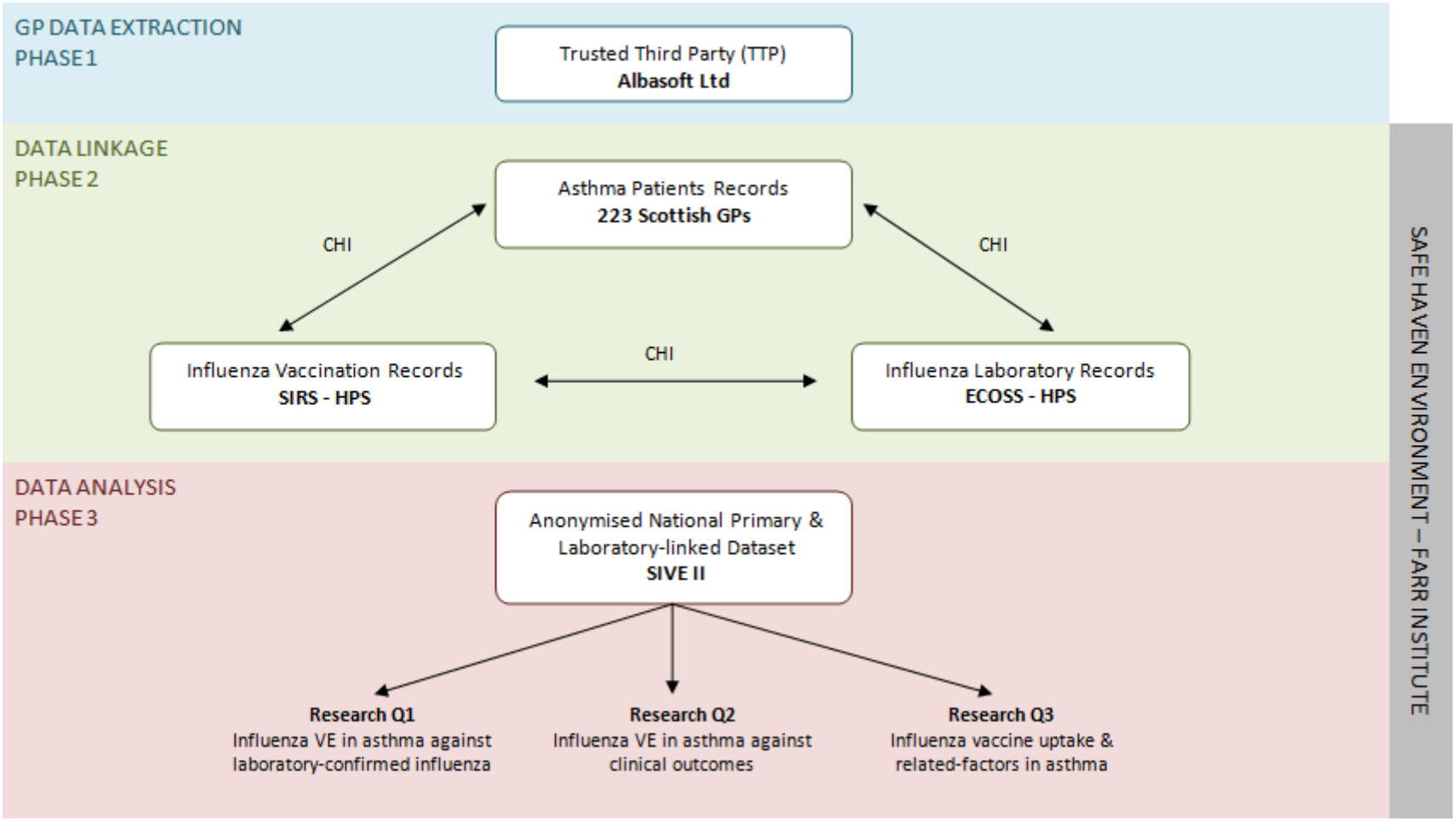

Figure 5.2: Phases of data extraction, linkage and analysis in a secured environment

5.2.4 Study period

This study included 14 influenza seasons (2000/01 to 2015/16). Specifically, all relevant data were used from 1 September 2000 to 31 August 2016. The unit of analysis was person-time for each individual with asthma that was alive and had a general practice registry. Each year was separated into four periods (see Figure 5.3). Influenza surveillance data were used to determine the start and end of each influenza season.(241) However, pre-specified dates were used to define the rest periods. Specifically, pre-influenza season starts in 1 September, post-influenza season ends in 31 May and non-influenza season ranges from 1 June to 31 August.(128)

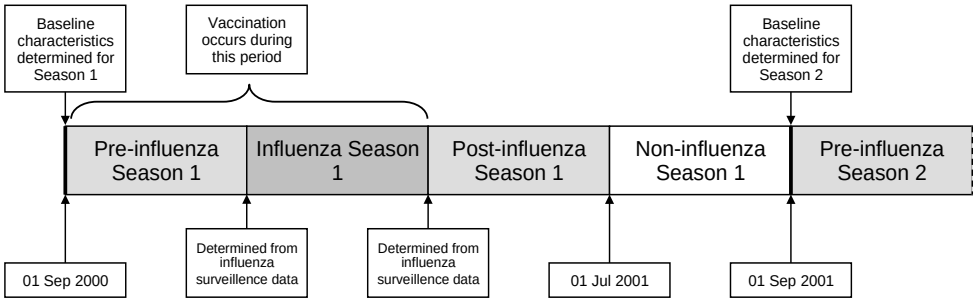

Figure 5.3: Division of each study year into four influenza season periods
Reproduced with permissions from: Simpson CR, et al. Evaluating the effectiveness, impact and safety of live attenuated and seasonal inactivated influenza vaccination: protocol for the Seasonal Influenza Vaccination Effectiveness II (SIVE II) study. BMJ Open 2017; 7(2): e014200.

5.2.5 Exposure definition

Influenza vaccines are provided free of charge in Scotland to any individual with a high-risk medical condition, such as asthma.(36) The administration is predominantly carried out in general practices. In addition, the CHI number and other data related to vaccine administration are recorded from the general practice in order to be compensated.(246) Vaccination records were also available from primary schools.(247) The latent period between influenza vaccine administration and serologic response is two weeks.(248) Thus, an individual is considered fully protected 14 days following vaccination. The exposure status was based on vaccination administrated between the start of the pre-influenza season and the end of the influenza season (see Figure 5.3). Individuals vaccinated from 1st of September until the end of the influenza season defined the "exposed" group. Those vaccinated between the start

of the influenza season and the 14th day following vaccination defined the "unexposed" group. Individuals with no vaccination record or being vaccinated after the being tested for influenza were classified as the "unexposed" group. Those tested for influenza and with a vaccination history of less than 14 days also defined the "unexposed" group.

5.2.6 Study outcomes

General practices that were part of the sentinel scheme were asked to obtain diagnostic nasal or nasopharyngeal swabs from patients that present ILI symptoms. Each general practice was required to collect up to five samples per week and submit to the West of Scotland Specialist Virology Centre (WoSSVC).(249) Each swab sample was tested by the WoSSVC using the multiplex PCR test for a number of respiratory pathogens, including influenza. Subtype and genetic characterisation was also performed of positive samples for influenza.

5.2.7 Population characteristics and confounding factors

A number of population characteristics were determined at the baseline of each influenza season, which referred to the first day of each pre-influenza season (1st September). These characteristics can potentially confound the relationship between influenza vaccine and infection and where included in the analyses if they were found (through statistical tests or based on epidemiologic knowledge) to confound the relationship between vaccination and influenza infection.

Sex at birth was included in a binary format (females and males). Age was split into eight categories based on eligibility for various vaccinations. Specifically, the 0-1 year group represented mainly infants where those less than six months old were not eligible for vaccination. The 2-4 age group included pre-school children who have been eligible to receive the live attenuated vaccine since 2013 at GPs. The 5-11 age group represented primary-school children eligible for the live attenuated vaccine at school. The 12-17 age group included secondary-school children where they can receive the live attenuated vaccine through their GPs. The age groups 18-44 and 45-64 included working adults who currently are not included in one of the at-risk groups eligible for a free vaccination in the UK.(36) The age groups 65-74 and $\geq$75 included older people that are at high risk of severe ILI according to the UK immunisation guidelines.(36) Socio-economic status (SES) was assessed using the Scottish Index of

Multiple Deprivation (SIMD). The SIMD classification is based on quintiles of deprivation: quintile 1 is the most deprived and quintile 5 is the least deprived. In addition, the SIMD combines 38 indicators across the following seven domains: income, employment, health, education, housing, geographical access to services and crime (see appendix A11; p 316).(250) Type of settlement was assessed using the urban/rural 8 fold classification (UR8). The UR8 is a standard definition of rural areas in Scotland; 1 is assigned to large urban areas, which corresponds to settlements of 125,000 or more people and 8 is assigned to remote rural areas, which corresponds to settlements with a population of less than 3,000 people, and with a drive time of over 60 minutes to a settlement of 10,000 or more.(251) Underlying chronic medical conditions other than asthma (respiratory disease such as COPD, chronic heart disease, chronic liver disease, chronic neurological disease, diabetes and immunosuppression) which increase the risk of influenza illness were also included this study.(246) The number of at-risk conditions (co-morbidities) was also included and presented in six risk groups; 1 was assigned to individuals with an additional medical condition other than asthma and 6 was assigned to those with six additional medical conditions. Receipt of influenza vaccine in the previous season was included in a binary format (yes or no). The location where the swab samples were collected was also presented in three categories: general practice, hospital and unknown.

5.2.8 Statistical analysis

Baseline characteristics of study participants were described. The relation between vaccination status and baseline characteristics was also provided for both case and control groups. Proportions and ORs were used to describe differences between study groups depending on the nature of each variable.(128) In addition, all baseline population characteristics were presented as categorical variables and the x^2 test was used to describe any association in relation to exposure or outcome. Any missing data between cases and controls was also reported.(128) All planned tests were two-tailed and the level of significance was set at $P<0.05$. All statistical analyses were carried out using RStudio (Version 1.0.143).(252)

Primary and secondary analysis

The primary analysis of this study was the provision of a pooled VE estimate across all seasons. Individual-level analysis was also performed separately for each of the 14

influenza seasons according to the published protocol.(128) All secondary analyses (subgroup and sensitivity) were focused on the post-pandemic influenza seasons (2010/11 to 2015/16). The number of swab samples was small in the pre-pandemic years (2000/10 to 2008/09), which prohibited the amount of analyses that could be carried out for these years.

Individual-level subgroup analysis for influenza A and B types and sub-types was performed for each influenza season.(128) Post-hoc analyses included stratification of the VE by age groups in order to investigate what age the VE begins declining in adults due to the phenomenon of immunosenescence. VE estimates for the new childhood LAIV by influenza strain was also explored to assess if the vaccine provides higher protection due to its composition. Stratified VE analysis by previous vaccination history was also conducted to explore any impact of previous influenza vaccination. The effect of previous influenza vaccination on the current vaccination estimates was assessed using one previous season. This was because I was only interested in the recent effects of vaccination. In addition, there are methodological challenges in the evaluation of repeated vaccination effects in more than one prior influenza season. Specifically, studies which have looked more than one previous year have ended up coding individuals as never vaccinated, always vaccinated and sometimes vaccinated as the two biggest groups are never and always vaccinated.(253) Thus, the inclusion of one previous influenza season was sufficient and allowed to investigate the interaction between previous and current vaccination. VE estimates when influenza A(H3) and A(H1N1) strains dominated were also conducted to increase the power of the analysis and provide more precise VE estimates for these common circulating strains. Finally, I stratified the VE by swab sample location to explore if the there are differences between primary (sentinel or non-sentinel) and secondary care settings that could affect the VE. Sensitivity analysis was also conducted comparing pooled VE estimates between sentinel and non-sentinel health care facilities for influenza surveillance.(128)

Vaccine effectiveness

The VE was estimated comparing the vaccine status between cases and controls. Patients with ILI symptoms and a positive test for influenza were defined as cases, while those who had consulted with ILI symptoms and who had a negative test for influenza were defined as controls. Patients were considered as vaccinated if the data of vaccination was two weeks or more before the swab sample was taken. While, if the vaccination was performed less or within two weeks before a swab sample was taken this was defined as unvaccinated.

VE and 95% CIs were estimated based on adjusted ORs).(128) ORs were calculated by the regression coefficients of the vaccine status in the model. A generalised additive logistic regression model was used to explain the relationship between our binary outcome (influenza infection) and our predictor (influenza vaccine) in presence of other confounding covariates. I used a logistic regression model because of the dichotomous format of my study outcome. In addition, the flexibility of the generalised additive models meant that I could fit most of my data even with the presence of nonlinear relationships and significant noise in the predictor variables.(254) However, it is not recommended to over-fit the data despite the flexibility of these models. This is because a complex model (with high number of degrees of freedom) would be difficult to be replicated in other datasets. The model can also become too tailored in order to fit data from all the included covariates and random noise in the sample rather than reflecting the true effect estimate in the overall population.

The sample of my study was not large enough to support an overly complex model with all potential confounding covariates included. The presence of missing values in some of my study covariates (e.g. SIMD) could also not support a complex model. Finally, only a few covariates are associated with influenza positivity. Thus, a parsimonious (or less-complex) approach was employed to fit my study data and develop the model for the provision of the adjusted VE estimates. The aim of this approach is to fit the best model for the available data by initially including all covariates in the model and then exclude those covariates that are not statistically or

clinically significant. However, covariates that may not be statistically, but clinically related with the outcome can be still included in the final model.

Specifically, in my study I followed the two-stage step indicated by this parsimonious approach in order to build my final model for the provision of the adjusted VE estimates. First, I included all potential confounding covariates and then I removed all the statistically non-significant covariates. However, I still kept the age covariate in my model as it is widely accepted in the literature that it can confound the relationship between influenza vaccination and infection. Thus, the model which provided VE estimates was adjusted for the effects of the covariates: time, age, underlying medical conditions and the source of swab sample collection which were either statistically or epidemiologically associated with the outcome. Adjustment for time was performed for the provision of the adjusted, but also of the unadjusted VE estimates.

Time was included as a spline function to model the baseline rate of influenza activity for every season. This method helped correct for any bias related to the number of patients with influenza positive and influenza negative tests at different time periods during a season. In September the influenza positivity is usually very low and peaks in December at around 10-20% during seasonal and 40% in pandemic years. In February to March the influenza positivity decreases to around 5%. Therefore, the epidemic curve of the influenza virus is not a symmetric or a uniform curve and its pattern differs from season to season. Thus, the spline function and to an extent the generalised additive regression models are able to describe the epidemic curve of the influenza virus regardless of its pattern every season due to their high flexibility. The overall aim of the spline function and the respective adjustment for time in the model was to avoid the introduction of bias. Specifically, bias can result from the fact that at the end of each season more people will have a test positive for influenza and at the same time a higher number of vaccinated individuals will be observed compared to the beginning of the season. Thus, the spline function prohibits any bias related to time differences related to influenza circulation and vaccine administration.

Meta-analysis for pooled VE estimates for all influenza types/subtypes

Pooled VE estimates for all influenza A and B strains were also produced using the generic inverse variance method for meta-analysis. The standard χ^2 test and the I^2 statistic were used to determine statistical heterogeneity for pooled VE estimates. Forest plots were used to display any statistically heterogeneity for pooled VE across multiple seasons. Pooled and individual-season ORs and their 95% CIs were provided using the adjusted ORs and their intervals (at a logarithm scale) already calculated in the subgroup analyses (see Table 5.4 and Table 5.5) for all influenza strains as input. In case of heterogeneity the effect estimates from the random-effects model were chosen due to variability of each influenza season. Thus, each vaccine effect estimate from each influenza season was considered as an estimate coming from a different 'study' justifying the use of random-effects models over fixed-effects models.

5.2.9 Sample size and power calculation

A sample size of 1.25 million patients was included in the SIVE II study from 223 general practices. In Scotland, the Pandemic Influenza Primary Care Reporting (PIPER) 2014/15 cohort had 263,000 individuals.(128) PIPER is surveillance system for influenza in Scotland which routinely extracted pre-existing primary care related to ILI or ARI and influenza or pneumococcal vaccination.(241) Thus, PIPER enabled the timely provision of trends on influenza activity and vaccine effectiveness (including the 2009/10 pandemic).(241) The size of the SIVE II study is five times larger than in PIPER 2014/15 cohort giving a multiplier ratio of about 5:1. There were 1,745 swab samples in the PIPER 2014/15 cohort and this gives 8,725 (1,745*5) swab samples for the SIVE II study per year.(128) If the annual prevalence of asthma is around 8.0% in Scotland, then 1 in 12 individuals have asthma.(255) Thus, I expected 727 (8,725/12) patients with asthma swabbed every year as about 8.0% (or 1 in 12) of the Scottish population has asthma and 1,454 (727*2) swab samples for ILI would have been taken from asthma patients in the final two seasons. Assuming that 582 or 40.0% (1,454*0.40) of the asthma patients had been vaccinated for influenza and the number of tests positive for influenza was 218 or 15.0% (1,454*0.15), this gave an 80.0% power to detect a VE of 33.0%.

5.2.10 Ethics and permissions

Permissions were obtained from the Privacy Advisory Committee of the Information Services Division, NHS Scotland [68/14], the National Research Ethics Committee West Midlands – Edgbaston [15/WM/0035], the National Caldicott Guardian and General Practice Data Custodians.

5.2.11 Reporting checklists for observational epidemiological studies

I used the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) and REporting of studies Conducted using Observational Routinely-collected Data (RECORD) checklists to guide the reporting of this TND case-control study (see Appendix A12-A13; p 317-319).(256)(257)

5.3 Results

5.3.1 Study population characteristics

A total of 6,921 swab samples were taken from 5,824 asthma patients presenting (of a total registered primary care asthma population of 1,830,772 person-seasons). These swabs were carried out in primary or secondary care settings on people with a diagnosis of asthma with ILI symptoms over 14 influenza seasons and then tested for influenza infection with real-time RT-PCR test (see Figure 5.4).

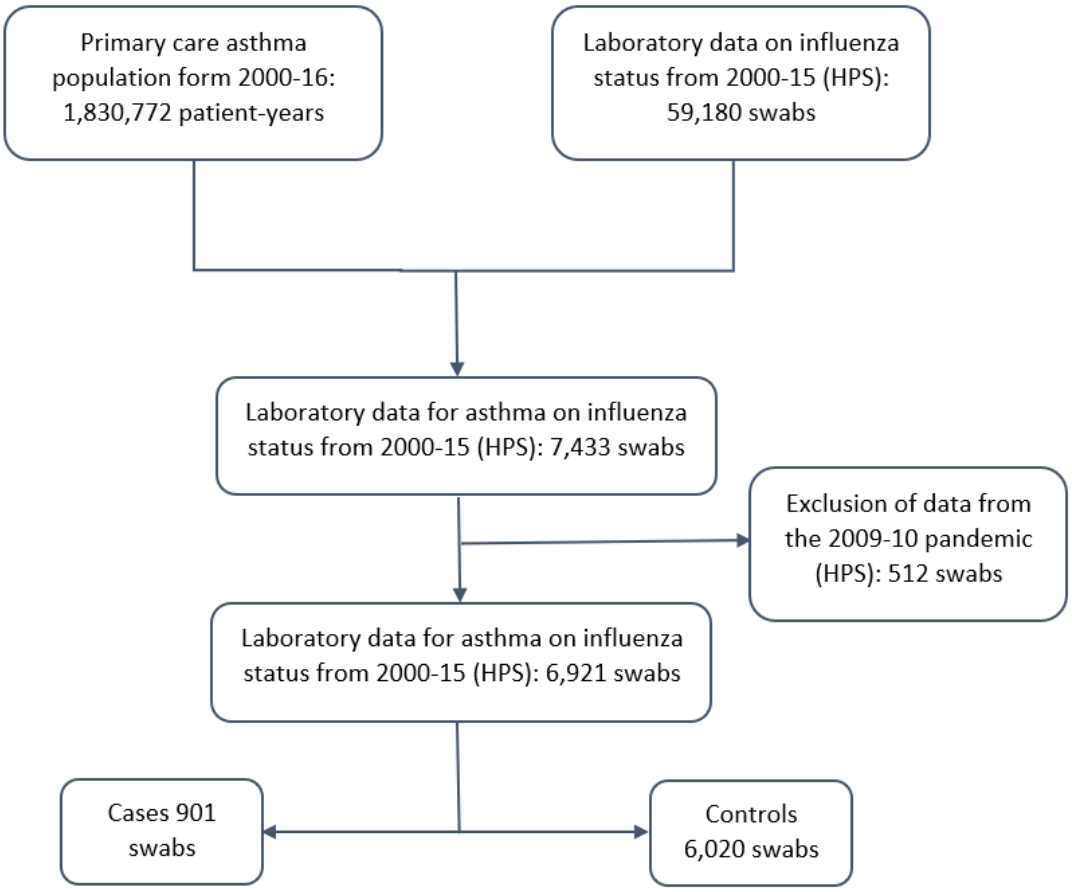

Figure 5.4: Flow diagram for the test-negative design case-control study for an asthma population for the influenza seasons 2000/01 to 2015/16, Scotland, UK

Of the 6,921 swab samples included in the study population, 901 (13.0%) tested positive for influenza and classified as cases (see Table 5.1). Out of the 6,921 total collected swab samples, 6,020 (87%) were negative for influenza and were classified as controls.

Table 5.1: Number of influenza (sub)types out of the 901 influenza positive cases

Influenza (sub)types	No. of influenza (sub)types / No. of cases (%)
Influenza A	677/901 (74.0)
A(H1N1)	276/901 (30.6)
A(H3)	228/901 (25.3)
A(unknown)	173/901 (19.2)
Influenza B	229/901 (25.4)
Influenza A & B	<10/901 (0.6)

All subgroups were represented in the study. However, swab samples were more likely to be from adults less than 65 years old, female, and socioeconomically deprived patients with cardiopulmonary conditions such as COPD, chronic heart disease and diabetes and living in large urban areas. Swab samples were also more often collected during secondary care consultations (hospital) than in a primary care setting.

Demographic characteristics and vaccination status of the study populations are provided in Table 5.2. During the study, 13% of swab samples were positive for RT-PCR-confirmed influenza. Patients more likely to test positive for influenza were aged 12-44 years, lived in remote small towns, with no previous season influenza vaccine and had a swab sample collected from a primary care setting. There was no difference between cases and controls in sex, socioeconomic deprivation and at-risk chronic diseases (COPD, heart disease, liver disease, neurological disease, diabetes, immunosuppression). The vaccination coverage was higher in women, in older adults ($\geq$65 years old), in those least deprived (5^{th} quintile), in large urban areas, with at-risk chronic conditions such as COPD, heart disease, neurological disease and diabetes, in those with the most co-morbidities, in those with an influenza vaccine in the previous year and in those with consultation at a hospital (see Table 5.2).

Table 5.2: Baseline characteristics for cases and controls with asthma for 14 influenza seasons, Scotland, 2000-16 (n=6,921 swabs)

Covariates	Total samples	No. Vaccinated at test (% of total)	P value*	No. of positive swabs (% of total)	P value	Swab-positive adjusted OR[a]	Adjusted 95% CI
Gender							
Female (ref)	4139	1939(46.8)	0.003	529(12.8)	0.5	NA	NA
Male	2782	1204(43.3)		372(13.4)		1.05	0.9 to 1.2
Age group (years) [b]							
0-1	11	3(27.3)	<0.001	1(9.1)	0.4	0.7	0.04 to 3.6
2-4	219	72(32.9)		23(10.5)		0.8	0.5 to 1.3
5-11	661	233(35.2)		73(11.0)		0.9	0.6 to 1.2
12-17	517	134(25.9)		69(13.3)		1.1	0.8 to 1.5
18-44	2014	504(25.0)		275(13.7)		1.1	0.9 to 1.4
45-64	1827	923(50.5)		256(14.0)		1.1	0.9 to 1.4
65-74	806	595(73.8)		94(11.7)		0.9	0.7 to 1.2
≥75 (ref)	865	678(78.4)		110(12.7)		NA	NA
Deprivation quintile[c]							
1[d] (ref)	1672	710(42.5)	0.03	219(13.1)	0.5	NA	NA
2	1721	798(46.4)		209(12.1)		0.9	0.7 to 1.1
3	1201	567(47.2)		172(14.3)		1.1	0.9 to 1.4
4	1119	506(45.2)		143(12.8)		1.0	0.8 to 1.2
5	1055	507(48.1)		130(12.3)		0.9	0.7 to 1.2
Urban/rural score[e]							
1 (ref)	3802	1732(45.6)	0.006	433(11.4)	<0.001	NA	NA
2	1720	749(43.5)		247(14.4)		1.3	1.1 to 1.5
3	448	198(44.2)		71(15.8)		1.5	1.1 to 2.0
4	91	40(44.0)		16(17.6)		1.7	0.9 to 2.8
5	66	25(37.9)		17(25.8)		2.7	1.5 to 4.6
6	502	265(52.8)		65(12.9)		1.2	0.9 to 1.5
7	69	27(39.1)		16(23.2)		2.3	1.3 to 4.0
8[f]	123	66(53.7)		17(13.8)		1.2	0.7 to 2.0

COPD	834	561(67.3)	<0.001	98(11.8)	0.2	0.9	0.7 to 1.1
Chronic heart disease	774	565(73.0)	<0.001	95(12.3)	0.5	0.9	0.7 to 1.2
Chronic liver disease	113	57(50.4)	<0.001	15(13.3)	0.9	1.0	0.6 to 1.7
Chronic neurological disease	373	263(70.5)	<0.001	46(12.3)	0.7	0.9	0.7 to 1.3
Diabetes	638	447(70.1)	<0.001	79(12.4)	0.6	0.9	0.7 to 1.2
Immunosupression	182	90(49.5)	0.3	20(11.0)	0.4	0.8	0.5 to 1.3
Number of risk groups (comorbidities)							
1 (ref)	4512	1608(35.6)	<0.001	598(13.3)	0.7	NA	NA
2	1150	675(58.7)		149(13.0)		1.0	0.8 to 1.2
3	763	500(65.5)		97(12.7)		1.0	0.8 to 1.2
4	350	254(72.6)		39(11.1)		0.8	0.6 to 1.1
5	121	87(71.9)		13(10.7)		0.8	0.4 to 1.4
6	25	19(76.0)		5(20.0)		1.6	0.5 to 4.1
Influenza vaccine in previous season							
Yes	3624	2604(71.9)	<0.001	422(11.6)	<0.001	0.8	0.7 to 0.9
No (ref)	3297	539(16.3)		479(14.5)		NA	NA
Swab samples taken in general practices or hospitals							
General practice (ref)	1884	628(33.3)	<0.001	266(14.1)	0.03	NA	NA
Hospital	5010	2494(49.8)		628(12.5)		0.9	0.7 to 1.0
Unknown	27	21(77.8)		7(25.9)		2.1	0.8 to 4.9

Abbreviations: OR: odds ratio; CI: confidence interval; NA: not applicable
a Adjusted for gender, age and socioeconomic deprivation
b Age group available for 6,920 swabs
c Deprivation score only available for 6,768 swabs
d Most socioeconomically deprived
e Urban/rural score only available for 6,821 swabs
f Remote rural areas
* All p-values were estimated using the x^2 test

5.3.2 Vaccine effectiveness

The protective effects of the vaccination are provided below into the following order: a) overall VE estimates for each pre- and post-pandemic season, b) influenza strain specific VE estimates for each post-pandemic season, c) pooled VE estimates (meta-analyses) for influenza strains circulating in the post-pandemic seasons, d) pooled VE estimates across all post-pandemic seasons stratified by age group and influenza strain, e) pooled VE estimates from post-pandemic seasons where either the influenza A(H1N1) or A(H3N2) strains predominated, f) pooled VE estimates across all post-pandemic seasons stratified by previous and current season vaccination history, g) pooled VE estimates from 2013/14 to 2015/16 seasons stratified by vaccine type in children, and h) pooled VE estimates stratified by influenza swab sample location.

Overall, there were 901 cases who were influenza positive, and 6,020 controls who were influenza negative. The pooled VE was 49.4% (95% CI: 39.7 to 57.5) in the asthma population across all 14 influenza seasons (2000/01 to 2015/16).

Pre-pandemic influenza seasons

The VE for the seasons 2000/01, 2001/02 and 2002/03 could not be estimated as there were only a total of 78, 59 and 76 swabs from people with asthma with less than 10 positive swabs in each season and none of the cases had been vaccinated. The VE for the influenza seasons 2003/04 to 2007/08 were estimated, but the estimates were imprecise (wide 95% CIs) due to low number of cases (<20) and the low number of swabs from people who were vaccinated (<5). Similarly, the adjusted VE could not be estimated for the season 2006/07 due to low numbers. In the 2008/09 season the VE was -227.7% (95% CI: -667.4 to -39.9) and significant. Although there was a higher vaccine uptake amongst cases compared to controls which resulted in this negative VE estimate, the imprecise estimate was due to the low number of cases.

Post-pandemic influenza seasons

2010/11: The VE in the first post-pandemic season 2010-11 was 76.1% (95% CI: 55.6 to 87.1). This season was characterised by a large proportion of positive swabs (n=123/487, 25.3%) and A(H1N1) being the predominating circulating strain. Vaccine uptake was over 20.0% higher in the control group compared to cases.

2011/12: No significant VE was observed for the 2011/12 season which was 45.1% (95% CI: -35.1 to 77.7). Only 4.9% of the total samples were positive for influenza and the vaccine uptake was higher amongst cases.

2012/13: The VE was 45.2% (95% CI: 13.8 to 65.1) for the 2012/13 season where all influenza types and subtypes co-circulated and a high number of samples tested positive for influenza (n=143/834, 17.2%) was observed.

2013/14: In the 2013/14 season the adjusted VE was significant and up to 52.3% (95% CI: 6.5 to 75.6) where the H1N1 strain predominated. The CIs were, however, wide due to the small number of positive samples during that season (n=54/932, 5.8%).

2014/15: During the season 2014/15 a significant VE of 48.6% (95% CI: 27.8 to 63.4) was found despite the higher number of vaccinated individuals amongst cases. The H3N2 strain predominated and a substantial higher influenza positivity was observed (n=232/1413, 16.4%) than in the previous season. Additional subgroup analyses (which will be described below) revealed that the VE was due to influenza B which contributed to an overall positive and significant estimate.

2015/16: In the last season 2015/16 a higher and significant VE of 57.8% (95% CI: 40.1 to 70.3) estimate was observed compared to the previous season. However, the number of tests positive for influenza were slightly lower (n=201/1670, 12.0%) compared to the previous season and the H1N1 strain was the predominant circulating strain (see Table 5.3).

Table 5.3: Vaccine effectiveness for laboratory-confirmed influenza and predominant circulating influenza by season, Scotland, 2000-16, (n=6,921)

Season	Total sample	Influenza-positive (cases)		Influenza-negative (controls)		Total positive (%)	Unadjusted vaccine effectiveness[a] (95% CI)	Adjusted vaccine effectiveness[b] (95% CI)	Dominant circulating strain(s)
		Vaccinated / total (n)	Vaccinated (%)	Vaccinated/ total (n)	Vaccinated (%)				
All seasons	6,921	354/901	39.3	2,789/6,020	46.3	13.0	46.4* (37.1 to 54.3)	49.4*[b] (39.7 to 57.5)	NA
2000/1	78	0/6	0.0	22/72	30.6	7.7	100 (-Inf to 100)	100 (-Inf to 100)	A/New Caledonia/20/99 (H1N1) B/Beijing/184/93
2001/2	59	0/3	0.0	11/56	19.6	5.1	100 (-Inf to 100)	100 (-Inf to 100)	A/Panama/2007/99 (H3N2)
2002/3	76	0/3	0.0	17/73	23.3	4.0	100 (-Inf to 100)	100 (-Inf to 100)	A/Panama/2007/99 (H3N2) B/Hong Kong/330/01
2003/4	123	1/15	6.7	24/108	22.2	12.2	54.4 (-304.6 to 94.9)	34.9 (-599.1 to 93.9)	A/Fujian/411/2002 (H3N2)
2004/5	99	4/16	25.0	24/83	28.9	16.2	36.2 (-145.6 to 83.4)	27.4 (-201.0 to 82.5)	A/Wellington/01/2004 (H3N2) B/Shanghai/361/2002
2005/6	90	2/11	18.2	28/79	35.4	12.2	74.0 (-44.8 to 95.3)	-46.0 (-1286.2 to 84.6)	B/Malaysia/2506/2004
2006/7	75	2/7	28.6	15/68	22.1	9.3	16.7 (-513.5 to 88.7)	0.00 (-Inf to 100.)	A/Wisconsin/67/05 (H3N2)

2007/8	87	1/9	11.1	19/78	24.4	10.3	77.1 (-118.1 to 97.6)	76.3 (-170.5 to 97.9)	A/Solomon Island/3/2006 (H1N1)
2008/9	324	18/50	36.0	81/274	29.6	15.4	-57.0 (-207.2 to 19.7)	-227.7 (-667.4 to -39.9)	A/Brisbane/10/2007 (H3N2)
2010/11	487	29/123	23.6	176/364	48.4	25.3	70.1 (49.5 to 82.3)	76.1 (55.6 to 87.1)	A/California/07/2009 (H1N1)pdm2009 B/Brisbane/60/2008
2011/12	574	14/28	50.0	241/546	44.1	4.9	34.4 (-44.3 to 70.1)	45.1 (-35.1 to 77.7)	A/Victoria/208/2009 (H3N2)
2012/13	834	50/143	35.0	323/691	46.7	17.2	48.2 (22.2 to 65.5)	45.2 (13.8 to 65.1)	A/Victoria/208/2009 (H3N2) A/St Petersburg/27/2011 (H1N1) B/Brisbane/60/2008 B/Brisbane/3/2007 B/Massachusetts/02/2012
2013/14	932	26/54	48.2	457/878	52.1	5.8	37.7 (-10.7 to 64.9)	52.3 (6.5 to 75.6)	A/California/07/2009 (H1N1)pdm09
2014/15	1413	122/232	52.6	605/1181	51.2	16.4	36.3 (13.3 to 53.2)	48.6 (27.8 to 63.4)	A/Texas/50/2012 (H3N2) B/Yamagata/16/88
2015/16	1670	85/201	42.3	746/1469	50.8	12.0	54.8 (37.8 to 67.1)	57.8 (40.1 to 70.3)	A/California/07/2009 (H1N1)pdm09 B/Brisbane/60/2008

Abbreviations: CI: confidence interval; NA: not applicable
a Adjusted for time (i.e. days) only
b Adjusted for time (i.e. days), age, number of risk groups, swab location (i.e. GP or hospital)
-VE estimates for the seasons 2000/01 to 2008/09 were adjusted only for time and age while the rest seasons were adjusted for all variables mentioned in the b annotation.

5.3.3 Subgroup and sensitivity analyses

Vaccine effectiveness by influenza type and subtype

The VE for the most common influenza A and B circulating strains was estimated for each influenza season where possible. The VE for the influenza seasons 2000/01 to 2002/03 for various influenza types and subtypes was not estimated. The small number of swab samples in these seasons prohibited the estimation of the overall VE against any influenza infection, thus any further exploration was abandoned. VE estimates for the pre-pandemic seasons (2003-08) against influenza A and B types/subtypes were estimated for this thesis (see appendix A14; p 327). However, all additional analyses were focused on post-pandemic seasons (2010-15) where the sample size was adequate and the vaccine records were more accurately recorded for further exploration.

In the first post-pandemic season (2010/11), the overall VE was significant mainly against influenza A(H1N1) subtype and B with estimates 70.7% (95% CI: 32.5 to 87.3) and 83.17% (95% CI: 44.3 to 94.9), respectively. In 2011/12, the VE against any influenza strain was not significant due to small number of cases resulting in very imprecise estimates for the A(H3) subtype and no estimate for the A(H1N1) subtype. The VE against influenza A was 51.9% (95% CI: 18.2 to 71.8) in 2012/13 season where all influenza A subtypes co-dominated. A particularly high VE up to 77.5% (95% CI: 9.8 to 94.4) was observed against A(H1N1), but no significant VE for co-circulating subtype A(H3) and type influenza B (see Table 5.4). The overall VE for 2013/14 was significant, but no significant VEs were observed for any influenza A or B type or subtype. The overall positive VE was likely from influenza A and its A(H1N1) subtype which had a positive non-significant VE, imprecision being due to low swab positivity for that season (due to low circulating influenza). In 2014/15, an overall significant VE was found with a high swab positivity (16.4%). This was likely driven by the significant VE of 77.0% (95% CI: 53.9 to 88.5) found for influenza B which was one of the predominant circulating strains. In 2015/16, the overall VE was higher than other strain specific estimates and the swab positivity was up to 12.0%. A lower VE of 52.3% (95% CI: 27.5 to 68.6) against influenza A was observed. No significant VE was found for influenza A subtypes. VE estimate of 54.7% (95% CI: 19.5 to 74.5) was also observed against influenza B (see Table 5.5).

Table 5.4: Vaccine effectiveness for laboratory-confirmed influenza (sub)types by season, Scotland, 2010-13

Dominant circulating strain(s) for each influenza season	Influenza types & subtypes	Influenza-positive (cases)		Influenza-negative (controls)		Total positive (%)	Unadjusted vaccine effectiveness[a] (95% CI)	Adjusted vaccine effectiveness[b] (95% CI)
		Vaccinated/ total (n)	Vaccinated (%)	Vaccinated/ total (n)	Vaccinated (%)			
Season: 2010-2011 A/California/07/2009 (H1N1)pdm2009 B/Brisbane/60/2008	Influenza A & B	29/123	23.6	176/364	48.4	25.3	70.1 (49.5 to 82.3)	76.1 (55.6 to 87.1)
	Influenza A	24/98	24.5	181/389	46.5	20.1	63.1 (33.5 to 79.5)	69.4 (37.9 to 84.9)
	A(H3)	0/0	0.0	205/487	42.1	0.0	0.0 (-Inf to 100)	0.0 (-Inf to100)
	A(H1N1)	17/79	21.5	188/408	46.1	16.2	68.8 (37.9 to 84.3)	70.7 (32.5 to 87.3)
	Influenza B	5/26	19.2	200/461	43.4	5.3	78.0 (37.3 to 92.3)	83.2 (44.3 to 94.9)
Season: 2011-2012 A/Victoria/208/2009 (H3N2)	Influenza A & B	14/28	50.0	241/546	44.1	4.9	34.4 (-44.3 to 70.1)	45.1 (-35.1 to 77.7)
	Influenza A	12/23	52.2	243/551	44.1	4.0	27.0 (-74.6 to 69.5)	40.2 (-68.4 to 78.8)
	A(H3)	6/11	54.6	249/563	44.2	1.9	20.1 (-173.0 to 76.6)	3.7 (-240.5 to 75.0)
	A(H1N1)	0/0	0.0	255/574	44.4	0.0	0.0 (-Inf to 100)	0.0 (-Inf to 100)
	Influenza B	2/5	40.0	253/569	44.5	0.9	57.1 (-186.7 to 93.6)	71.8 (-358.1 to 98.3)
Season: 2012-2013	Influenza A & B	50/143	35.0	323/691	46.7	17.2	48.2 (22.2 to 65.5)	45.2 (13.8 to 65.1)
	Influenza A	32/91	35.2	341/743	45.9	10.9	43.8	51.9

A/Victoria/208/2009 (H3N2) A/St Petersburg/27/2011 (H1N1) B/Brisbane/60/2008 B/Brisbane/3/2007 B/Massachusetts/02/2012							(9.6 to 65.1)	(18.2 to 71.8)
	A(H3)	17/45	37.8	356/789	45.1	5.4	27.9 (-36.3 to 61.9)	38.0 (-25.7 to 69.4)
	A(H1N1)	3/17	17.7	370/817	45.3	2.0	79.8 (28.3 to 94.3)	77.5 (9.8 to 94.4)
	Influenza B	18/53	34.0	355/781	45.5	6.4	40.0 (-9.8 to 67.3)	11.7 (-70.7 to 54.3)

Abbreviations: CI: confidence interval; NA: not applicable

a Adjusted for time (i.e. days) only

b Adjusted for time (i.e. days), age, number of risk groups, swab location (i.e. GP or hospital)

-There are cases with unknown influenza A subtype which explains why the total influenza A(H3) and A(H1N1) samples do not add exactly to the total influenza A samples

Table 5.5: Vaccine effectiveness for laboratory-confirmed influenza (sub)types by season, Scotland, 2013-16

Dominant circulating strain(s) for each influenza season	Influenza types & subtypes	Influenza-positive (cases)		Influenza-negative (controls)		Total positive (%)	Unadjusted vaccine effectiveness[a] (95% CI)	Adjusted vaccine effectiveness[b] (95% CI)
		Vaccinated/ total (n)	Vaccinated (%)	Vaccinated/ total (n)	Vaccinated (%)			
Season: 2013-2014 A/California/07/2009 (H1N1)pdm09	Influenza A & B	26/54	48.2	457/878	52.1	5.8	37.7 (-10.7 to 64.9)	52.3 (6.5 to 75.6)
	Influenza A	24/50	48.0	459/882	52.0	5.4	39.0 (-11.0 to 66.4)	49.8 (-0.6 to 74.9)
	A(H3)	2/6	33.3	481/926	51.9	0.6	65.9 (-105.0 to 94.3)	-3.9 (-1304.5 to 92.3)
	A(H1N1)	18/34	52.9	465/898	51.8	3.7	21.4 (-59.2 to 61.2)	32.0 (-52.2 to 69.6)
	Influenza B	2/5	40.0	481/927	51.9	0.5	45.2 (-259.1 to 91.7)	100 (0 to 100)
Season: 2014-2015 A/Texas/50/2012 (H3N2) B/Yamagata/16/88	Influenza A & B	122/232	52.6	605/1181	51.2	16.4	36.3 (13.3 to 53.2)	48.6 (27.8 to 63.4)
	Influenza A	103/184	56.0	624/1229	50.8	13.0	21.2 (-12.0 to 44.5)	30.4 (-2.0 to 52.5)
	A(H3)	79/140	56.4	648/1273	50.9	9.9	21.1 (-16.0 to 46.4)	26.4 (-12.0 to 51.6)
	A(H1N1)	5/6	83.3	722/1407	51.3	0.4	-290.9 (-3301.3 to 55.1)	-157.0 (-2565.5 to 75.2)
	Influenza B	20/49	40.8	707/1364	51.8	3.5	62.0 (30.3 to 79.3)	77.0 (53.9 to 88.5)
Season: 2015-16	Influenza A & B	85/201	42.3	746/1469	50.8	12.0	54.8 (37.8 to 67.1)	57.8 (40.1 to 70.23)

A/California/07/2009 (H1N1)pdm09 B/Brisbane/60/2008	Influenza A	60/135	44.4	771/1535	50.2	8.1	45.1 (20.1 to 62.2)	52.3 (27.5 to 68.6)
	A(H3)	2/6	33.3	829/1664	49.8	0.4	39.0 (-294.0 to 90.5)	78.1 (-102.6 to 97.6)
	A(H1N1)	51/104	49.0	780/1566	49.8	6.2	32.8 (-2.0 to 55.7)	36.7 (-0.6 to 60.2)
	Influenza B	26/67	38.8	805/1603	50.2	4.0	60.9 (33.9 to 76.8)	54.7 (19.5 to 74.5)

Abbreviations: CI: confidence interval; NA: not applicable

a Adjusted for time (i.e. days) only

b Adjusted for time (i.e. days), age, number of risk groups, swab location (i.e. GP or hospital)

-There are cases with unknown influenza A subtype which explains why the total influenza A(H3) and A(H1N1) samples do not add exactly to the total influenza A samples

Pooled vaccine effectiveness for influenza types and subtypes (meta-analysis)

The overall VE estimate was 55.0% (95% CI: 44.0 to 63.0) against influenza A and B types as it is shown by the OR provided in the random-effects model below. Heterogeneity for this pooled estimate was detected, but it was small and non-significant (see Figure 5.5). Lower pooled VE of 47.0% (95% CI: 34.0 to 57.0) against influenza A type. However, no heterogeneity was observed for this type (see Figure 5.6).

A substantially lower VE estimate of 29.0% (95% CI: 1.0 to 49.0) was detected for the influenza A(H3) subtype. The pooled VE point estimate was also not significant, but no heterogeneity was shown (see Figure 5.7). A higher pooled VE of 48.0% (95% CI: 19.0 to 67.0) was found against the influenza A(H1N1) subtype compared to the A(H3) subtype as it shown by the OR of the random effects model. However, low-to-moderate non-significant heterogeneity was observed across seasons (see Figure 5.8). The highest pooled VE was 62.0% (95% CI: 32.0 to 79.0), detected for influenza B subtype according to the OR of the random-effects model. A Higher heterogeneity of 52.0% (but non-significant) was also observed for the influenza B compared to other types and subtypes. In addition, the OR appears zero during the 2013/14 season. This happened due to low circulating levels of influenza B strains resulting in small to zero OR which prohibited the provision of any meaningful OR in the meta-analysis and a subsequent VE estimation (see Figure 5.9).

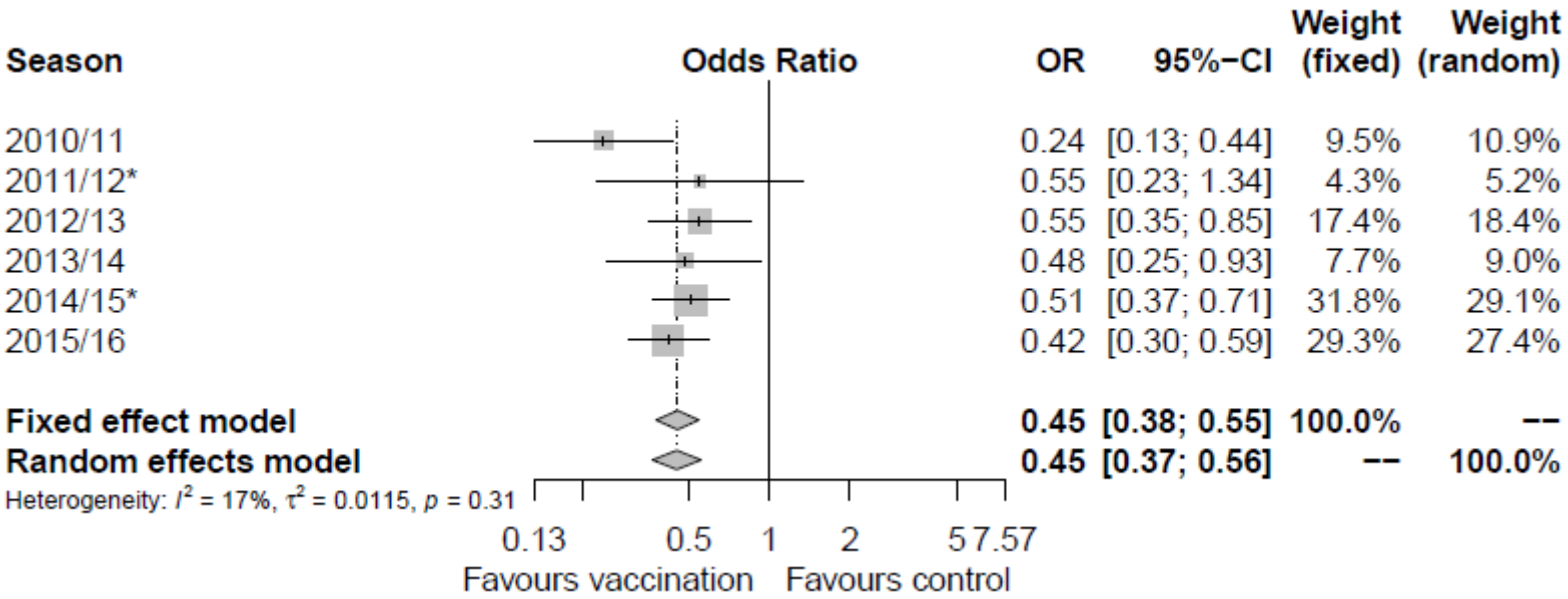

Figure 5.5: Vaccine effectiveness against laboratory confirmed overall influenza (influenza A and B) by season
*****Poorly vaccine matched seasons

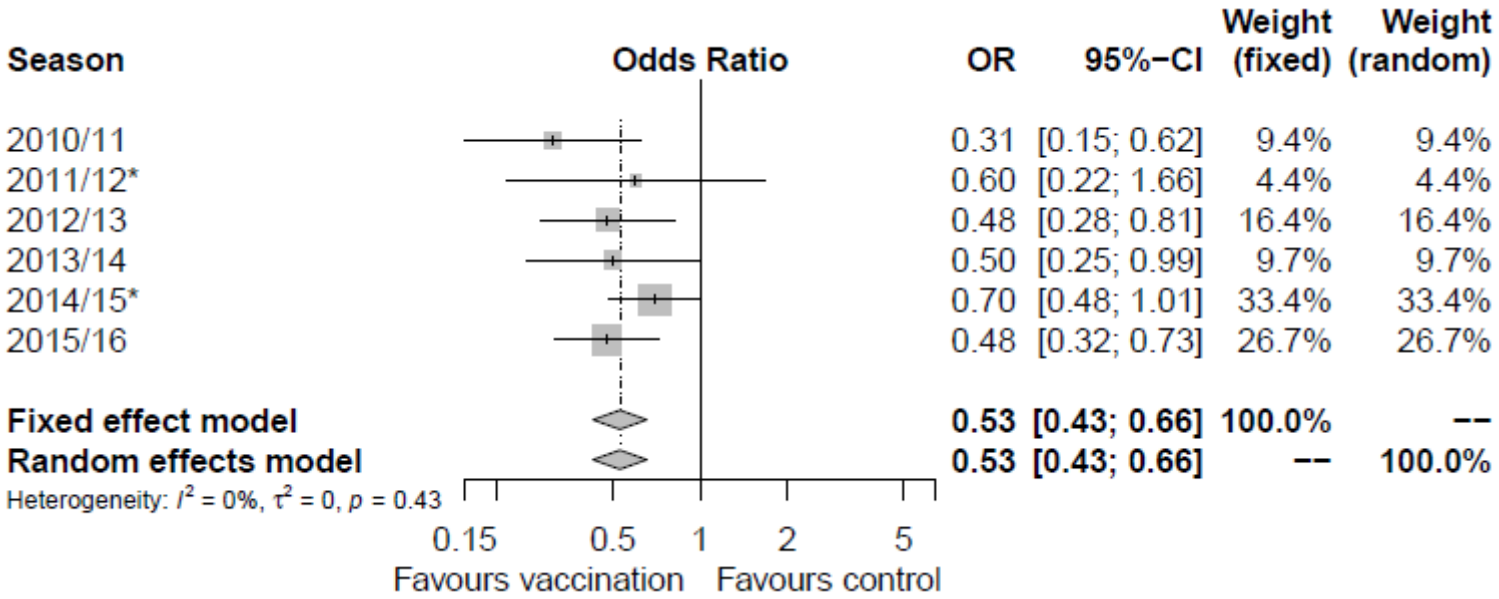

Figure 5.6: Vaccine effectiveness against laboratory confirmed influenza A type by season
***Poorly vaccine matched seasons**

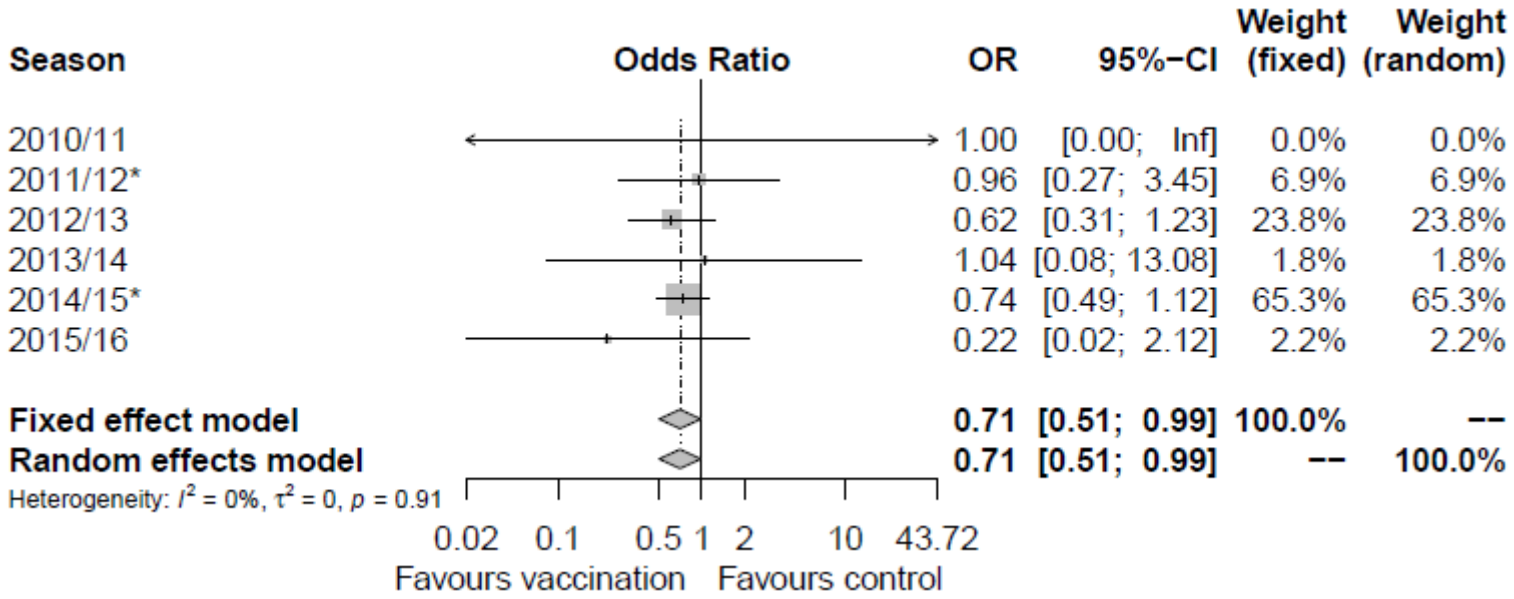

Figure 5.7: Vaccine effectiveness against laboratory confirmed influenza A(H3) subtype by season
***Poorly vaccine matched seasons

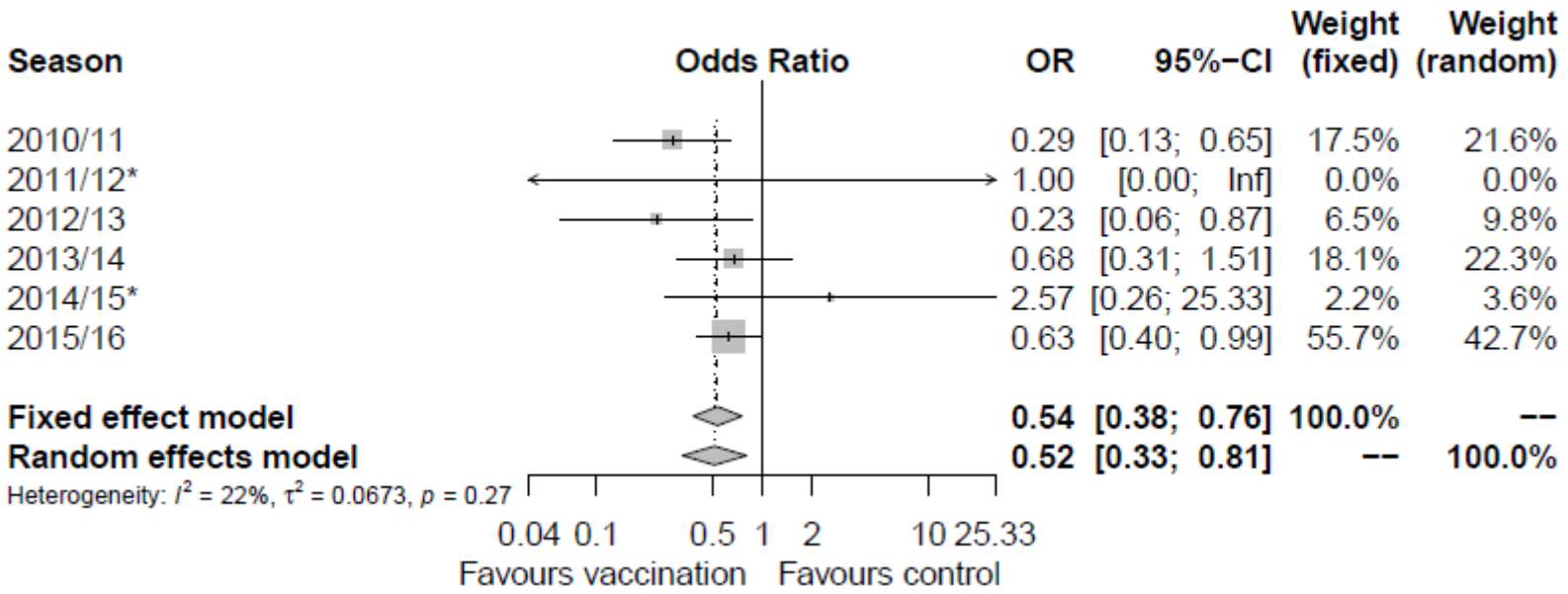

Figure 5.8: Vaccine effectiveness against laboratory confirmed influenza A(H1N1) subtype by season
***Poorly vaccine matched seasons

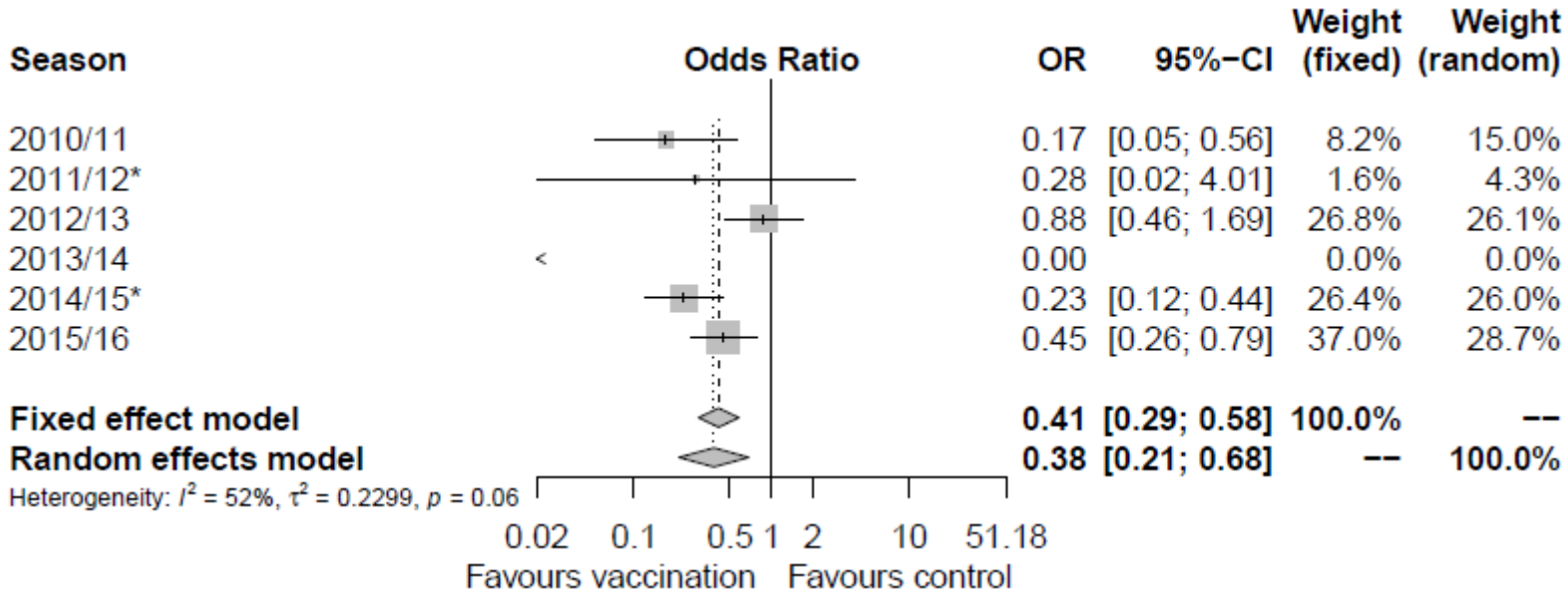

Figure 5.9: Vaccine effectiveness against laboratory confirmed influenza B subtype by season
*****Poorly vaccine matched seasons

Vaccine effectiveness by age group

When an analysis was performed by age group (in order to investigate the age where immunosenescence begins) in relation to VE. This analysis showed that the VE was low in those 55 years and above against influenza A and its subtypes (except 65-74 year old for type A), while significantly positive high VEs for influenza B were found. VE was high in people with asthma under 18 years of age, with a non-significant VE against A(H1N1) of 90.5% (95% CI: -45.4 to 99.4) (see Table 5.6).

Table 5.6: Vaccine effectiveness for laboratory-confirmed influenza by various age groups, Scotland, 2010-16

Age (years)	Influenza types & subtypes	Influenza-positive (cases)		Influenza-negative (controls)		Total positive (%)	Unadjusted vaccine effectiveness[a] (95% CI)	Adjusted vaccine Effectiveness[b] (95% CI)
		Vaccinated/ total (n)	Vaccinated (%)	Vaccinated/ total(n)	Vaccinated (%)			
All ages	Influenza A & B	326/781	41.7	2548/5129	49.7	13.2	48.6 (39.2 to 56.6)	55.0 (45.8 to 62.7)
	Influenza A	255/581	43.9	2619/5329	49.2	9.8	39.7 (26.9 to 50.3)	48.1 (35.8 to 58.1)
	A(H3)	106/208	51.0	2768/5701	48.5	3.5	26.0 (-0.8 to 45.6)	33.8 (6.7 to 53.1)
	A(H1N1)	94/240	39.2	2780/5670	49.0	4.1	43.2 (23.6 to 57.8)	46.6 (25.4 to 61.8)
	Influenza B	73/205	35.6	2801/5705	49.1	3.5	59.0 (44.2 to 69.9)	61.5 (45.7 to 72.7)
≤17	Influenza A & B	31/101	30.7	368/974	37.8	9.4	52.9 (23.4 to 71.0)	46.0 (11.2 to 67.2)
	Influenza A	19/59	32.2	380/1016	37.4	5.5	45.9 (0.6 to 70.5)	41.6 (-9.1 to 68.7)
	A(H3)	8/26	30.8	391/1049	37.3	2.4	55.7 (-11.0 to 82.3)	51.1 (-25.4 to 80.9)
	A(H1N1)	4/15	26.7	395/1060	37.3	1.4	64.9 (-66.8 to 92.6)	90.5 (-45.4 to 99.4)
	Influenza B	12/45	26.7	387/1030	37.6	4.2	69.6 (26.1 to 87.5)	56.3 (3.8 to 80.2)
18-54	Influenza A & B	94/376	25.0	733/2093	35.0	15.2	54.0 (39.2 to 65.2)	57.0 (42.3 to 68.0)
	Influenza A	73/288	25.4	754/2181	34.6	11.7	50.5 (32.4 to 63.8)	53.4 (35.3 to 66.5)

	A(H3)	22/84	26.2	805/2385	33.8	3.4	58.4 (28.4 to 75.8)	53.3 (17.9 to 73.5)
	A(H1N1)	33/143	23.1	794/2326	34.1	5.8	45.7 (14.4 to 65.5)	53.0 (23.8 to 71.1)
	Influenza B	22/89	24.7	805/2380	33.8	3.6	49.9 (15.9 to 70.1)	54.9 (21.1 to 73.7)
55-64	Influenza A & B	51/104	49.0	384/667	57.6	13.5	51.1 (22.0 to 69.4)	57.6 (29.6 to 74.5)
	Influenza A	44/80	55.0	391/691	56.6	10.4	28.8 (-20.7 to 58.0)	33.9 (-16.6 to 62.5)
	A(H3)	18/29	62.1	417/742	56.2	3.8	2.6 (-145.9 to 61.4)	2.1 (-178.5 to 65.6)
	A(H1N1)	17/33	51.5	418/738	56.6	4.3	38.0 (-36.3 to 71.8)	38.7 (-43.4 to 73.8)
	Influenza B	7/24	29.2	428/747	57.3	3.1	78.7 (45.0 to 91.8)	88.2 (61.2 to 96.4)
65-74	Influenza A & B	61/91	67.0	488/656	74.4	12.2	54.8 (22.5 to 73.6)	56.8 (24.0 to 74.9)
	Influenza A	49/71	69.0	500/676	74.0	9.5	49.8 (5.3 to 73.3)	50.8 (5.3 to 74.4)
	A(H3)	18/24	75.0	531/723	73.4	3.2	-13.4 (-249.3 to 63.2)	1.0 (-196.9 to 67.0)
	A(H1N1)	22/30	73.3	527/717	73.5	4.0	57.5 (-37.4 to 86.9)	60.5 (-37.9 to 88.7)
	Influenza B	12/20	60.0	537/727	73.9	2.7	65.3 (9.0 to 86.9)	65.8 (5.2 to 87.6)
≥75	Influenza A & B	89/109	81.7	575/739	77.8	12.9	48.9 (4.8 to 72.5)	51.9 (9.2 to 74.5)
	Influenza A	70/83	84.3	594/765	77.7	9.9	25.2 (-53.4 to 63.5)	28.0 (-50.6 to 65.6)
	A(H3)	40/45	88.9	624/803	77.7	5.3	-13.5	-15.4

							(-232.9 to 61.3)	(-278.2 to 64.8)
	A(H1N1)	18/19	94.7	646/829	77.9	2.2	-542.3 (-6752.7 to 39.8)	-501.0 (-5639.5 to 37.1)
	Influenza B	20/27	74.1	644/821	78.4	3.2	67.6 (15.1 to 87.6)	70.4 (19.8 to 89.1)

Abbreviations: CI: confidence interval

a Adjusted for time (i.e. days) only

b Adjusted for time (i.e. days), age, number of risk groups, swab location (i.e. GP or hospital)

Vaccine effectiveness for seasons with A(H1N1) predominate strain

Post-pandemic seasons with the same dominant influenza A subtype were also pooled for the provision of overall and influenza strain specific VE estimates. This analysis aimed to increase the power to identify more precise VE estimates for the A(H1N1) and A(H3) subtypes as they usually do not co-dominate every season. In addition, the pooling of seasons with the same influenza A dominant subtype would decrease the heterogeneity between seasons due to differences in circulating viral strains. Thus, the seasons 2010/11, 2013/14 and 2015/16 were pooled when the A(H1N1) was the predominant strain. The total VE was 60.4% (95% CI: 47.8 to 70.0) and 45.3% (95% CI: 21.8 to 61.7) against the A(H1N1) strain. No significant VE was found against the A(H3) strain since it circulated at low levels during those seasons. High VE of 62.9% (95% CI: 38.5 to 77.6) was, however, found against influenza B which co-dominated with the A(H1N1) strain in 2010/11 and in 2015/16 seasons (see Table 5.7).

Vaccine effectiveness for seasons with A(H3N2) predominate strain

Pooled VE of 47.8% (95% CI: 32.6 to 59.6) was also provided when the A(H3) strain dominated in 2011-13 and 2014/15 seasons. Significant VE was found for both influenza A and B. However, the adjusted VE for influenza A(H3) was significant despite higher vaccine uptake amongst cases. Further investigation with a larger sample size is needed to explore this unexplained VE in our data (see Table 5.8).

Table 5.7: Vaccine effectiveness for laboratory-confirmed influenza with A(H1N1) the predominant circulating strain in 2010/11, 2013/14 and 2015/16, Scotland (n=3,089)

Influenza types & subtypes	Influenza-positive (cases)		Influenza-negative (controls)		Total positive (%)	Unadjusted vaccine effectiveness[a] (95% CI)	Adjusted vaccine effectiveness[b] (95% CI)
	Vaccinated/ Total (n)	Vaccinated (%)	Vaccinated/ Total (n)	Vaccinated (%)			
Influenza A & B	140/378	37.0	1379/2711	50.9	12.2	56.5 (44.5 to 66.0)	60.4 (47.8 to 70.0)
Influenza A	108/283	38.2	1411/2806	50.3	9.2	48.5 (32.0 to 61.0)	55.3 (38.7 to 67.4)
A(H3)	4/12	33.3	1515/3077	49.2	0.4	53.1 (-69.4 to 87.0)	69.3 (-33.6 to 92.9)
A(H1N1)	86/217	39.6	1433/2872	49.9	7.0	42.1 (20.6 to 57.8)	45.3 (21.8 to 61.7)
Influenza B	33/98	33.7	1486/2991	49.7	3.2	64.4 (44.2 to 77.4)	62.9 (38.5 to 77.6)

Abbreviations: CI: confidence interval; NA: not applicable

a Adjusted for time (i.e. days) only

b Adjusted for time (i.e. days), age, number of risk groups, swab location (i.e. GP or hospital)

Table 5.8: Vaccine effectiveness for laboratory-confirmed influenza with A(H3) the predominant circulating strain in 2011-13 and 2014/15, Scotland (n=2,821)

Influenza types & subtypes	Influenza-positive (cases)		Influenza-negative (controls)		Total positive (%)	Unadjusted vaccine effectiveness[a] (95% CI)	Adjusted vaccine effectiveness[b] (95% CI)
	Vaccinated/ Total (n)	Vaccinated (%)	Vaccinated/ Total (n)	Vaccinated (%)			
Influenza A & B	186/403	46.2	1169/2418	48.4	14.3	39.9 (24.1 to 52.4)	47.8 (32.6 to 59.6)
Influenza A	147/298	49.3	1208/2523	47.9	10.6	30.0 (8.6 to 46.3)	40.0[c] (19.7 to 55.2)
A(H3)	102/196	52.0	1253/2625	47.7	7.0	23.2 (-5.6 to 44.1)	29.8 (0.1 to 50.7)
A(H1N1)	8/23	34.8	1347/2798	48.1	0.8	50.7 (-18.6 to 79.5)	57.0 (-19.0 to 84.5)
Influenza B	40/107	37.4	1315/2714	48.5	3.8	53.7 (29.3 to 69.6)	61.4 (38.2 to 75.9)

Abbreviations: CI: confidence interval; NA: not applicable

a Adjusted for time (i.e. days) only

b Adjusted for time (i.e. days), age, number of risk groups, swab location (i.e. GP or hospital

c The VE for influenza A may be explained by the adjusted VE for Influenza A unknown subtype, adjVE: 39.7 (95%CI: -0.4 to 63.8) and raw numbers (46.8% vaccinated cases vs 48.1 vaccinated controls and total positive 2.8%)

Effect of current and prior season vaccination

The effect of previous season vaccination on the current season was also explored by combining previous and current vaccination histories compared with individuals with no vaccination history. The VE was lower in those vaccinated in both seasons than those vaccinated in the current season only. However, the point VE estimates did not differ significantly as evidenced from their overlapping confidence intervals. In addition, no significant VE was found for those vaccinated in the previous, but not in the current season (see Table 5.9).

Table 5.9: Vaccine effectiveness of the combined influenza vaccinations in the previous and current season, Scotland, 2010-16 (n=5,910)

Vaccination status		Unadjusted vaccine effectiveness[a] (95% CI)	Adjusted vaccine effectiveness[b] (95% CI)	P-value compared with unvaccinated in both seasons[b]
Previous season	Current season			
Unvaccinated	Unvaccinated	0.0 (0.0 to 0.0)	0.0 (0.0 to 0.0)	NA
Vaccinated	Unvaccinated	3.7 (-26.7 to 26.8)	-4.0 (-66.8 to 35.0)	0.9
Unvaccinated	Vaccinated	60.8 (43.3 to 73.0)	83.2 (57.5 to 93.3)	0.0002
Vaccinated	Vaccinated	46.9 (36.2 to 55.9)	55.1 (34.4 to 69.2)	<0.001

Abbreviations: CI: confidence interval; NA: not applicable
a Adjusted for time (i.e. days) only
b Adjusted for time (i.e. days), age, number of risk groups, swab location (i.e. GP or hospital)

Vaccine effectiveness of LAIV versus TIV

For the sub-group analysis VE in children for the LAIV and the TIV vaccine from 2013/14 to 2015/16, significant VE for LAIV against influenza B was found. There was no other significant VE for any other viral strain or from the TIV (see Table 5.10).

Table 5.10: Vaccine effectiveness for laboratory-confirmed influenza (sub)types by influenza vaccine type in children (≤17 years old), Scotland, 2013-16 (n=613)

Influenza types & subtypes	Influenza vaccines*	Influenza-positive (cases)		Influenza-negative (controls)		Total positive (%)	Unadjusted vaccine effectiveness[a] (95% CI)	Adjusted vaccine Effectiveness[b] (95% CI)
		Vaccinated/ Total (n)	Vaccinated (%)	Vaccinated/ Total (n)	Vaccinated (%)			
Influenza A & B	TIV	9/38	23.7	118/460	25.7	7.6	51.2 (-11.0 to 78.5)	48.7 (-18.8 to 77.8)
	LAIV	10/39	25.6	105/447	23.5	8.0	44.9 (-24.3 to 75.6)	37.8 (-44.0 to 73.1)
Influenza A	TIV	5/24	20.8	122/474	25.7	4.8	56.4 (-25.9 to 84.9)	53.4 (-36.7 to 84.1)
	LAIV	9/28	32.1	106/458	23.1	5.8	-0.2 (-144.6 to 58.9)	-7.9 (-174.2 to 57.5)
A(H3)	TIV	3/11	27.3	124/487	25.5	2.2	41.7 (-146.0 to 86.2)	33.3 (-186.2 to 84.4)
	LAIV	3/11	27.3	112/475	23.6	2.3	24.32 (-224.78 to 82.37)	20.6 (-269.3 to 82.9)
A(H1N1)	TIV	1/6	16.7	126/492	25.6	1.2	59.6 (-264.8 to 95.5)	23.2 (-772.3 to 93.2)
	LAIV	3/8	37.5	112/478	23.4	1.7	1.2 (-353.2 to 78.4)	-16.2 (-656.7 to 82.2)
Influenza B	TIV	4/15	26.7	123/483	25.5	3.0	65.6 (-80.6 to 93.5)	85.3 (-22.2 to 98.3)
	LAIV	1/12	8.3	114/474	24.1	2.5	96.8* (57.5 to 99.8)	96.4* (45.9 to 99.8)

Abbreviations: CI: confidence interval; NA: not applicable; TIV: trivalent inactivated vaccine; LAIV: live attenuated influenza vaccine
*No vaccine is the reference group
a Adjusted for time (i.e. days) only
b Adjusted for time (i.e. days), age, number of risk groups and swab location (i.e. GP or hospital)

Vaccine effectiveness by location of swab sample

The number of swab samples from general practices was 873 samples compared to 4,710 from hospitals. There were no significant differences in VE estimates as evidenced by their overlapping confidence intervals (see Table 5.11). Samples from general practices were further investigated by comparing VE estimated between sentinel and non-sentinel practices. Similar sentinel practice VE was found, but no significant VE for non-sentinel practices due to small number of samples and the higher vaccine uptake in cases (see Table 5.12).

A sensitivity analysis was also performed by excluding swab samples collected from sentinel general practices. 704 of the total 5,910 virological tests were collected from sentinel general practices. The VE was similar to the overall VE in our primary analysis (see Table 5.13).

Table 5.11: Vaccine effectiveness for laboratory-confirmed influenza by swab sample location (general practice vs hospital), Scotland, 2010-16 (n=5,883)

Swab sample source	Influenza-positive (cases)		Influenza-negative (controls)		Total positive (%)	Unadjusted vaccine effectiveness[a] (95% CI)	Adjusted vaccine Effectiveness[b] (95% CI)
	Vaccinated/ total (n)	Vaccinated (%)	Vaccinated/ total(n)	Vaccinated (%)			
General practice	55/146	37.7	304/727	41.8	16.7	39.1 (7.2 to 60.1)	49.3 (18.4 to 68.5)
Hospital	266/628	42.4	2228/4382	50.8	12.5	50.4 (40.1 to 58.9)	55.4 (45.2 to 63.7)

Abbreviations: CI: confidence interval
a Adjusted for time (i.e. days) only
b Adjusted for time (i.e. days), age, number of risk groups
-There were n=27 swab samples where the source was coded as unknown

Table 5.12: Vaccine effectiveness for laboratory-confirmed influenza by swab sample location (sentinel vs non-sentinel GP), Scotland, 2010-16 (n=873)

Swab sample source	Influenza-positive (cases)		Influenza-negative (controls)		Total positive (%)	Unadjusted vaccine effectiveness[a] (95% CI)	Adjusted vaccine Effectiveness[b] (95% CI)
	Vaccinated/ total (n)	Vaccinated (%)	Vaccinated/ total(n)	Vaccinated (%)			
Sentinel GPs	35/102	34.3	249/602	40.2	14.5	46.2 (13.2 to 66.7)	45.6 (7.9 to 67.9)
Non-sentinel GPs	20/44	45.5	55/125	44.0	26.0	-6.4 (-160.9 to 56.6)	27.5 (-94.3 to 72.9)

Abbreviations: CI: confidence interval; GP: general practice
a Adjusted for time (i.e. days) only
b Adjusted for time (i.e. days), age, number of risk groups

Table 5.13: Vaccine effectiveness for laboratory-confirmed influenza by excluding swab samples from sentinel GP, Scotland, 2010-16 (n=5,206)

Swab sample source	Influenza-positive (cases)		Influenza-negative (controls)		Total positive (%)	Unadjusted vaccine effectiveness[a] (95% CI)	Adjusted vaccine Effectiveness[b] (95% CI)
	Vaccinated/total (n)	Vaccinated (%)	Vaccinated/ total(n)	Vaccinated (%)			
GP & hospital	326/781	41.7	2548/5129	49.7	13.2	48.6 (39.2 to 56.6)	55.0 (45.8 to 62.7)
No sentinel GPs	291/679	42.9	2299/4527	50.8	13.0	48.5 (38.2 to 57.0)	54.1 (44.0 to 62.4)

Abbreviations: CI: confidence interval; GP: general practice
a Adjusted for time (i.e. days) only
b Adjusted for time (i.e. days), age and number of risk groups

5.4 Discussion

Summary of main findings

During 14 influenza seasons (2000/01 to 2015/16), influenza vaccination reduced laboratory-confirmed influenza in those people presenting to and being swabbed in general practice and hospital settings by 49.4% (95% CI: 39.7 to 57.7) in 5,824 children and adults with asthma. A similar overall protection (55.0%) was also observed during the post-pandemic seasons (2010/11 to 2015/16) in the asthma population. Higher protection was observed during seasons with good vaccine antigenic match and where the A(H1N1) strain dominated. VE was moderate in cases of influenza A(H1N1) (47.0%) and influenza B (62.0%) and was low in cases of influenza A(H3) (29.0%). Moderate and significant protection for all influenza strains was detected in the young adults (aged 18-54 years old). The highest VE (96.0%) was detected against influenza B in children $\leq$17 years old with a LAIV administration during the 2013/14 to 2015/16 influenza seasons.

Comparisons with existing literature

In this study, the highest VE was observed in the 2010/11 season which was characterised by high influenza activity predominated by the influenza A(H1N1) and B strains in the UK.(258) There was a good match between the circulating and vaccine strains justifying the high and significant protection against predominant circulating strains.(259) In contrast, low and non-significant VE was detected in the 2011/12 season probably due to low and late activity of the predominant A(H3N2) strain and its antigenic distance from the vaccine strain.(260) In addition, intra-seasonal VE waning against the A(H3N2) was observed in the UK resulting in an even lower VE estimate over the course of the season.(261) The 2012/13 season was a mixed season with all common strains co-circulating. However, high and significant protection was only found against the A(H1N1) strain. A finding also seen in another UK study in the same season.(262) The antigenic drift observed for the circulating influenza B in the UK and the substantial decline in the VE against the influenza A(H3N2) in the second trimester following the vaccinations could justify the absence of protection for these co-dominant circulating strains in this season.(262)(263)

The influenza activity during the 2013/14 season was low and prolonged. Influenza A(H1N1) dominated with a good match with the respective vaccine strain.(264) A moderate overall protection was observed in this study, but no protection was seen for specific influenza A and B strains. This was mainly due to extremely overall low number of positive for influenza swabs. In the 2014/15 season significant overall VE was observed in this study despite the observed mismatch between the predominant A(H3N2) strain with the strain included in the vaccine.(265) Our stratified analysis by influenza type and sub-type showed that the overall significant VE was due to high VE against influenza B. A similar finding also observed in the UK-wide study assessing the VE in 2014/15.(265) Moderate overall protection was seen in 2015/16. This result was consistent with the VE of 55% seen in the UK in the same season.(266) No protection was seen against the dominant circulating A(H1N1) strain, which antigenically matched the vaccine strain.(267) This is in contrast to the overall UK findings where the highest VE (55%) was found against A(H1N1).(266) Significant VE was observed against influenza B for the same season and this was confirmed also in the UK study (266) despite lineage mismatch with the vaccine strain.(266)

The LAIV showed consistently high VE against influenza B in this study. The high VE of the live vaccine has also been observed in other studies.(159)(197) No significant VE was observed for any of the influenza A type and its A(H1N1) and A(H3) sub-types. The number of swab samples was too small in this subgroup analysis to show any protection. Therefore, replication of these findings is required in a larger study with more than three seasons.

Our finding of a significant pooled VE when the A(H1N1) dominated could be explained by the absence of vaccine mismatch over the three seasons.(259)(264)(267) While the lower pooled VE when the A(H3N2) dominated could be due to vaccine mismatch in the two out of the three seasons and the intra-seasonal VE waning.(262)(264) It is common the vaccine protection against influenza A(H3N2) to be lower than influenza A(H1N1) and B.(84)(269)

Amongst children ($\leq$17 years old) significant protection was found only against B, while protective effects have been observed for both influenza B and A(H1N1) in other studies.(165)(173) A recent TND study in Canada also found a similar VE of 43.3%

in children 6-59 months old with asthma from 2010/11 to 2013/14.(235) Higher VE for all influenza strains was found in young adults 18-54 years old. Amongst older adults (aged ≥55 year old), no protection was found against any of the influenza A(H3) and A(H1N1) sub-types. Similarly, no protection was seen in a recent hospital-based study in older patients with asthma aged 65 years and above.(236) Although, no strain specific estimates were provided to allow comparison. The lack of significant positive VE amongst adults aged 55 and above may indicate immunosenescence, but further research is needed due to sample power issues in this study.

The effect of previous influenza vaccination on current VE has been assessed in previous studies due to conflicting evidence.(185)(270-273)In this study the point VE estimate was higher for those without a previous season vaccination compared to those with a vaccination in both seasons. This finding could be indicative of a negative impact of previous immunity on current VE. On the other hand, unmeasured confounding or patient characteristics (e.g. more severe asthma) could explain the lower VE in individuals with regular vaccination pattern.

Strengths and limitations

Strengths of this study include the objective marker for influenza diagnosis using the RT-PCR laboratory test minimising the risk of information bias. In addition, the TND minimises the risk of selection bias which may arise due to differential healthcare seeking behaviour between cases and controls by assessing only the prevention of the vaccine against medically-attended influenza infection. By pooling the VE assessment over six seasons the power of the study was increased, which allowed the provision of VE estimates for different virus sub(types), vaccine types and patient characteristics.

There also several limitations in this study. In this study the cohort of people with asthma was based on general practice ever asthma diagnosis. Therefore, the vaccine uptake was probably underestimated since it is only recommended to people with asthma on inhaled or oral medication or previous history of asthma exacerbations requiring hospital admission. The VE in this study assessed only the prevention of influenza which was determined by RT-PCR viral swab. However, vaccinated individuals may have also been benefited by having less severe influenza illness and a subsequent lower risk of a severe asthma attack. A single swab may not identify all

cases of true influenza and viral shedding can be variable and is affected by age, the site of the swab, and the point in the illness at which the swab is taken. Thus, vaccine protection provided by any decrease in influenza severity cannot be quantified using this study design.(126) There was inadequate power for some sub-group analyses (e.g. by vaccine type and some age groups) despite the inclusion of data on six seasons. Thus, results from these relatively underpowered subgroup analyses need to be interpreted with caution. Results from the post-hoc analyses also need careful interpretation since they were not pre-specified in the protocol of this study. Measured confounders (e.g. asthma severity status) and other unmeasured confounders could still have influenced the VE estimates. The confounding effect on VE derived from TND studies needs to be assessed in future research work. There could be miscoding of LAIV administration as LAIV may have been coded using an influenza vaccine parent code. This would mean LAIV would be labelled as TIV. This is due to the parent code being the most widely used code by GPs (i.e. before the introduction of the LAIV in 2013). In this scenario, the power to detect LAIV VE is reduced. Thus, the true VE of the LAIV should be assessed in future studies including more than three seasons.

Implications and conclusion

This study showed that the vaccination can prevent influenza infection in people with asthma. Although variation in VE amongst people with asthma was observed for circulating strains, vaccine types and across age group. These findings may inform vaccine research and the current vaccination programme. Healthcare providers and asthma patients will now have a better understanding of the benefits of the seasonal influenza vaccination which may lead to higher acceptance and adoption (in particular for those groups with better evidence of protection). Thus, evidence from this study reinforces the recommendation for annual seasonal vaccination for people with asthma. Progress may be being made towards vaccines with better potency, durability which convey better protection.(110) Monitoring of VE should still be continued. Better sample size and sufficiently long follow-up periods though are required, particularly given the changes in the circulating viral strains and the seasonal vaccine formulations.

To conclude, this study provided compelling national evidence over a number of years that influenza vaccination can reduces the risk of influenza in people with asthma. Specifically, vaccinated individuals would have about 50% less risk of influenza infection compared to unvaccinated individuals. In addition, higher VE was observed in seasons with good antigenic match between the circulating and vaccine strains and against the A(H1N1) and B influenza strains.

5.5 Summary to Chapter 5

This study has shown that the influenza vaccines can prevent an important proportion of laboratory-confirmed influenza infection in people with asthma. The protection of the vaccines against clinical outcomes due to influenza infection also needs to be assessed. However, an exploratory analysis regarding differences in demographics between the vaccinated groups is necessary. The exploration of baseline differences between the vaccinated and unvaccinated individuals could discover significant differences and guide accordingly any planned analyses especially related to VE against clinical outcomes given the high presence of bias and confounding in these studies (see Chapter 4). Thus, in the following chapter a descriptive analysis of the characteristics of the asthma population derived from a primary care setting will be conducted in relation to vaccine uptake. Additionally, demographic differences between the immunisation groups will be explored for those having an emergency hospital admission for influenza or pneumonia.

Chapter 6. Demographic and clinical characteristics of influenza vaccinated and unvaccinated individuals with asthma in primary and secondary-care settings in Scotland: a vaccine uptake analysis

In the previous chapter, demographic and other differences between the study groups were described. However, the main aim of the study in Chapter 5 was to assess the association between the influenza vaccination and influenza infection. Thus, the description of the demographic or other characteristics of the asthma population was not fully explored. The aim of this chapter is to explore demographic characteristics of both vaccinated and unvaccinated people with asthma.

6.1 Introduction

People with chronic respiratory conditions such as asthma are eligible for influenza vaccination in most countries with an immunisation programme for influenza infection.(36) According to the UK guidelines people with *"asthma that requires continuous or repeated use of inhaled or systemic steroids or with previous exacerbations requiring hospital admission"* are eligible for influenza vaccination without cost to the individual.(36) Adults with asthma can be vaccinated with the trivalent split-virus inactivated vaccine, which is administered parenterally. Children with asthma aged 2-17 years are eligible for a quadrivalent LAIV which is administered nasally as a spray.(36) However, the "*LAIV is not recommended for children and adolescents with severe asthma or active wheezing, for example those who are currently taking oral steroids or who have been prescribed oral steroids in the last 14 days for respiratory disease*".(36) The benefits of the LAIV include: a) the ease of administration and acceptance by children and adolescents, b) better protection against influenza B strains (as it includes two influenza B strains) in naïve children with no previous exposure to variants of influenza B, c) cross-protection against new influenza B strains, d) immediate protection via innate immunity generated by local upper respiratory infection and e) indirect protection of the community by reducing the transmission of influenza to adults and particularly those with at-risk conditions for influenza.(274) In addition, a large UK study found that the LAIV administration in children would decrease the number of influenza infections in low-risk individuals than the vaccination of this low-risk individuals.(275) In the UK, despite long-standing influenza recommendations (over 30 years) for asthma and the introduction of

financial reimbursements to GPs that meet government immunisation targets, the uptake levels are still not in line with the WHO target of 75% vaccine uptake for all at-risk groups for influenza.(276) Suboptimal uptake trends have also been reported from across 44 European countries over a seven-year period. Vaccine uptake in patients with specific chronic conditions including asthma has been below 40% in most of these countries.(277)

In Scotland, the vaccine uptake in those <65 years and with conditions making at-risk to serious influenza illness has dropped over the years with the lowest uptake of 44.9% recorded during the season 2016/17.(278) However, the uptake in older people (aged >65 years old) is considerably higher with rates over 70.0%.(278) Similarly, an increased uptake pattern has been observed in children especially since the introduction of a universal childhood immunisation programme with the live vaccine in 2013. In primary school children (aged 4-11 years old) the uptake of the LAIV is even higher with a notable 73.0% coverage in 2016/17 in Scotland.(278) Thus, the observed low uptake in asthma, especially in adults less than 65 years compared to other age groups needs to be investigated.

The comparison of socio-demographic and clinical characteristics between vaccinated and unvaccinated groups could reveal which characteristics of the asthma population affect the propensity of being in either group. Thus, this analysis could help to improve the vaccine uptake by identifying the factors related to vaccination in the asthma population and maximise the impact of the influenza vaccination programme in Scotland. In addition, a detailed exploration of the characteristics in this population can guide the estimation of the VE especially against clinical outcomes, which suffer considerably from confounding and other bias.(279)

To the best of my knowledge, this is the first analysis in Scotland that aimed to explore characteristics between vaccinated and unvaccinated groups in the asthma population based on administrative data from general practice and emergency department hospital settings across 16 influenza seasons.

6.2 Methods

6.2.1 Study design

A vaccine uptake analysis was conducted to assess differences between vaccinated and unvaccinated individuals with asthma in the community and an emergency hospital admission due to influenza or pneumonia. Patients with asthma were identified from 223 primary care practices across Scotland (see sections 5.2.1 and 5.2.2 of Chapter 5). In this study, data from 194,319 patients (or 1,830,772 patient-seasons) were included from 16 influenza seasons (2000/01 to 2016/17). Data from each patient were analysed as person-time (see Table 6.1). It appears that the number of people with asthma increases from 2000/01 to 2016/17. This could simply reflect the nature of the cohort since a person that appears in 2000/01 can re-appear multiple times in the following years. In addition, this could be a combination of the asthma cohort surviving longer and due to an accumulation of people in the dataset with an asthma diagnosis as you - as you progress in years, the number of asthma patients increases and it almost doubles from 20001/01 to 2016/17 (see Table 6.1).

Table 6.1: Number of patients with an asthma diagnosis in the community per influenza season in Scotland

Influenza season	Asthma patients
2000/01	74,256
2001/02	80,075
2002/03	85,635
2003/04	90,671
2004/05	95,601
2005/06	100,011
2006/07	103,902
2007/08	107,579
2008/09	110,845
2009/10	112,676
2010/11	116,019
2011/12	118,717
2012/13	121,812
2013/14	124,293
2014/15	126,997
2015/16	129,768
2016/17	131,915

People with an asthma diagnosis in the community were also followed up in secondary healthcare settings to explore their demographic and health-related characteristics in relation to vaccine uptake. Specifically, there were in total 218,455 emergency hospital admissions during 16 influenza seasons in Scotland. Out of the 214,455 emergency admissions 5.3% were due to influenza or pneumonia (11,605/214,455). In this study the outcome of admission due to influenza or pneumonia was included rather than admission due to influenza only or influenza with an additional diagnosis of pneumonia because there was a low number for these types of admissions. Thus, the assessment of differences between vaccination groups would have been less informative if the admission was restricted to influenza only. Asthma was the main primary condition (or reason of admission) in 318 (2.7%) out of the 11,605 admissions due to influenza or pneumonia. However, asthma (or reason of admission) was a secondary condition in 23.2% (2,686) out of the 11,605 admissions for influenza or pneumonia (see Table 6.2).

Table 6.2: Emergency hospital admissions of asthma patients and in relation to influenza or pneumonia, Scotland, 2000/01 to 2016/17

Season	Total number of emergency admissions*	Emergency admissions due to influenza or pneumonia	Emergency admissions due to influenza or pneumonia with asthma as the main condition	Emergency admissions due to influenza or pneumonia with asthma as a secondary condition
All seasons	218,455	11,605/218,455 (5.3%)	318/11,605 (2.7%)	2,686/11,605 (23.2%)
2000/01	8,600	223/8,600 (2.6%)	5/223 (2.2%)	33/223 (14.8%)
2001/02	5,985	201/5,985 (3.4%)	6/201 (3.0%)	52/201 (25.9%)
2002/03	6,137	223/6,137 (3.6%)	7/223 (3.1%)	45/223 (20.1%)
2003/04	6,725	293/6,725 (4.4%)	10/293 (3.4%)	67/293 (22.9%)
2004/05	7,026	299/7,026 (4.3%)	4/299 (1.3%)	83/299 (27.8%)
2005/06	7,341	323/7,341 (4.4%)	4/323 (1.2%)	103/323 (31.9%)
2006/07	8,092	343/8,092 (4.2%)	7/343 (2.0%)	113/343 (32.9%)
2007/08	8,385	405/8,385 (4.8%)	4/405 (1.0%)	105/405 (25.9%)
2008/09	8,610	445/8,610 (5.2%)	4/445 (0.9%)	108/445 (24.3%)
2009/10	8,605	506/8,605 (5.9%)	21/506 (4.2%)	129/506 (25.5%)
2010/11	9,446	616/9,446 (6.5%)	21/616 (3.4%)	153/616 (24.8%)
2011/12	10,051	676/10,051(6.7%)	12/676 (1.8%)	161/676 (23.8%)
2012/13	10,305	716/10,305(6.9%)	14/716 (2.0%)	154/716 (21.5%)
2013/14	10,536	663/10,536(6.3%)	13/663 (2.0%)	134/663 (20.2%)
2014/15	10,766	862/10,766(8.0%)	32/862 (3.7%)	223/862 (25.9%)
2015/16	11,358	953/11,358(8.4%)	34/953 (3.6%)	222/953 (23.3%)
2016/17**	4,979	484/4,979 (9.7%)	18/484 (3.7%)	124/484 (25.6%)

* A patient with asthma can have more than one admission within the same season
** Data were not available for the full 2016/17 season as depicted by the lowest number of hospital admissions compared to previous seasons

Demographic and other characteristics related to vaccine uptake were determined in 8,231 admissions out of the 11,605 admissions. This is because only these 8,231 admissions were registered with a general practice in the same season of hospitalisation (the rest of the admissions being a patient is registered at a non-participating practice).

6.2.2 Population characteristics

Various demographic and clinical-related characteristics were assessed between vaccinated and unvaccinated asthma patients. Sex was considered as a dichotomous variable (female [baseline] versus male). Age was split into eight groups based on vaccine eligibility and other relevant information (see previous chapter for details). Socioeconomic status was assessed using the SIMD quintile. Urban/rural eight-fold score was also used (see previous chapter for details).

A number of other at-risk clinical conditions eligible for an influenza vaccine were also provided such as COPD, chronic heart disease, chronic liver disease, chronic neurological disease, diabetes and immunosuppression. The number of risk groups (comorbidities) was also provided in six categories. Where '1' was assigned to asthma patients with at least one comorbidity and '>5' to asthma patients with more than five comorbidities. Previous history of influenza vaccine for one season earlier was provided into a binary format (yes or no). The receipt of pneumococcal in the previous season was also provided into a binary format (yes or no). Smoking status was presented into four categories. Patients with no data on smoking status were included into the 'Not recorded' category. In addition, if a patient's smoking status was recorded for one season, but not in the follow-up seasons we assumed that the same status will exist in the future. Thus, the smoking status in the current year was carried over in the future seasons. Finally, when a patient appeared to have more than one smoking status within the same season priority was given to the smoking status that represents either current or previous history of smoking. For example, if a patient was a non-smoker and current smoker in the same season was assigned as a current smoker. Similarly, if a patient was current smoker and ex-smoker or non-smoker in the same season was assigned as a current smoker.

The Charlson Comorbidity Index in our data represents the weighted comorbidity score based on secondary care data in the previous five years.(280) The score for a patient was more reliable from the season 2006 and onwards as there was a five year history of data compared to the seasons before 2006. Thus, a patient with no comorbidities in the previous five years was assigned a zero score. The Charlson score in this study differs from the widely used Charlson Comorbidity Index which is produced based on a combination of the number and the severity or weight of each

comorbidity.(280) The number of consultations at a general practice for acute respiratory illness were also counted and presented in seven categories based on data in the previous season. Thus, patients with no previous year consultations were assigned into the zero category and those with a history of more than five consultations were assigned into the '>5' category. The number of previous season emergency hospital admissions was also provided into seven categories following a similar rigorous with the GP consultations.

The chronic underlying condition of the patients (asthma) would have been ideally explored through an asthma severity status. According to the British Thoracic Society (BTS) guidelines, asthma severity can be inferred by the amount of prescriptions needed to maintain good control of asthma-related symptoms (e.g. day-time, night-time and activity related symptoms) and maximise lung function.(281) Thus, the determination of the asthma severity status should be based on a holistic view of asthma status, which can be provided through a combination of information (e.g. asthma-related symptoms, asthma exacerbations, use of short-acting bronchodilators and tests to assess airway function and inflammation). However, most data in this study were available for asthma-related prescriptions such as bronchodilators, inhaled and oral corticosteroids. Thus, a variable reflecting a combination of asthma treatment options guided from the BTS 2016 guidelines was created as an indicator of asthma manifestations.(282) In the SIVEII study, medications for asthma were only available in a free-text format, which prohibited the manipulation of the data and the provision of the five treatment level options (previously referred as BTS steps) suggested in the BTS 2016 guidelines.(282) For example, some of the inhaled corticosteroids (ICS) doses were either missing or insufficiently recorded by the physicians. Thus, it was impossible to infer the exact dose for all ICS in our dataset and specify the dose of an ICS as low, medium or high.

We decided to treat the doses of the ICS in a binary format. A patient was assigned as having a 'high' versus 'not high' dose or a 'medium' versus 'not medium' dose based on a combination of age (<18 or $\geq$18 years old) and ICS formulation. Specifically, a patient not on Steps 1-3 was assigned to Step 0, children with a 'not medium' or 'not high' dose (e.g. older children) and adults with a 'not high' dose were assigned to Step

1, children with a 'medium' or 'high' dose (e.g. older children) and adults with a 'high' dose were assigned to Step 2 and children or adults that appeared in any of the previous three steps (Steps 0-2) and were also on oral steroids comprised Step 3.

The creation of the BTS step variable derived from GP free-text prescriptions issued in patients with asthma that were for asthma or other conditions. Thus, if a patient had multiple prescriptions within the same season priority was given to the highest BTS step and he was assigned to the highest BTS step. The BTS step variable in this study was thus created by combining steps suggested in the BTS 2016 guidelines (see Table 6.3 & 6.4).

Table 6.3: Treatment options (BTS steps) in the BTS 2016 guideline on the management of asthma

BTS treatment options (or steps)	**Asthma medications**
Step 0	Consider monitored initiation of treatment with low-dose ICS
Step 1	Low-dose ICS
Step 2	Add inhaled LABA to low-dose ICS
Step 3	Increased dose of ICS (low for children, medium for adults) & continue LABA & may add in other therapy (LTRA, theophylline, LAMA)
Step 4	Increased dose of ICS (medium for children, high for adults), add a fourth drug (LTRA, theophylline, beta agonist tablet, LAMA)
Step 5	Increased dose of ICS (medium for children, high for adults) and add OCS (frequent or continuous use, but consider other treatment to minimise use of OCS)
Abbreviations: BTS: British Thoracic Society; ICS: inhaled corticosteroids; OCS: oral corticosteroids; LTRA: leukotriene receptor antagonists; LAMA: long-acting muscarinic antagonists; LABA: long-acting beta agonists	

Table 6.4: BTS steps creation in this study based on the BTS 2016 guidelines

BTS steps	Study BTS steps	Asthma medications
Step 0	Step 0	A person not in the other steps (e.g. bronchodilators only)
Steps 1, 2 & 3	Step 1	Not medium dose of ICS for children; Not high dose of ICS for adults or children (e.g. older)
Step 4	Step 2	Medium dose of ICS for children; High dose of ICS for adults or children (e.g. older)
Step 5	Step 3	Step 0 & OCS; Step 1 & OCS; Step 3 & OCS; OCS only
Abbreviations: BTS: British Thoracic Society; ICS: inhaled corticosteroids; OCS: oral corticosteroids		

6.2.3 Data sources

Using the CHI number, individual-patient data were extracted from the 223 Scottish primary care practices and linked to a number of databases including primary care, vaccination records from SIRS and hospitalisation records. Details on the primary care and SIRS data are provided in sections 5.2.1 and 5.2.2 of Chapter 5. All linked databases and statistical analyses were hosted in the eDRIS (see Chapter 5 for further details).

Primary care: A separate dataset of prescriptions issued by GPs was used for the creation of the BTS step variable. The vaccine uptake status was determined only for those prescriptions issued to patients with an established asthma diagnosis in the community. The unit of analysis was the number of prescriptions per person (and not number of patient-seasons) between vaccinated and unvaccinated asthma patients for each BTS step across 15 seasons. My initial attempt to add the BTS step variable into the primary care cohort and the emergency hospital admissions datasets was abandoned due to high level of missing data. This was mainly due to a large number of unmatched records by patient ID and season. Specifically, many patient IDs from the dataset containing all the prescriptions could not be identified in the same season in the primary care or in the hospital dataset. As a result, more than 50% of prescriptions could not be allocated in the relevant BTS step. In addition, there were

no prescriptions data for the first two influenza seasons (2000/01 and 20001/02), while the primary care and hospital datasets included data from 2000 and onwards.

Scottish Morbidity Database: Individual-patient data from GPs were linked to the Scottish Morbidity Record (SMR), which contains episode level data on acute hospital admissions and death records of the Scottish population.(283) Specifically, the database contained four major linked datasets containing data on acute hospital admissions, psychiatric admissions, cancer registrations and death records from 1980 and onwards. The SMR01 dataset contains data on all general/acute inpatient and day cases.(284) The SMR01 dataset was constantly updated and held over 37 million records. All diseases and health problems were recorded in this database based on the 10th revision of the International Statistical Classification of Diseases and Related Health Problems (ICD-10) diagnostic code since 1996.(284) Regular quality assessments are carried out in both SMR01 hospital dataset. The quality of SMR01 data was high according to a recent report, where the accuracy rate was found over 90% during a 25 year data quality assessment.(285)

6.2.4 Statistical analysis

All demographic and clinical descriptive data were provided for both vaccine groups as percentages in tabulated format. Overall vaccine uptake between vaccinated and unvaccinated groups for each descriptive characteristic was also provided as a percentage. A univariate logistic regression analysis was performed to estimate any significant differences in vaccine uptake for each category of patients' characteristics. Adjusted ORs and their 95% CIs were provided for all categories. For the univariate logistic regression analysis, reference groups were usually those with the highest number. Missing values for each characteristic were also provided.

A multivariate correlation logistic regression model was performed to provide adjusted vaccine uptake effects from the influenza season 2000/01 to 2016/17. Specifically, the model aimed to provide ORs and 95% CIs estimates for each predictor of vaccine uptake adjusted for the effects of other covariates. The correlation model was able to adjust for the within patient correlation which occurred in the data. This is because the observations in this dataset were not independent as one asthma patient could appear in more than one season resulting in multiple observations of the same patient. Thus,

the observations from the same patient were correlated or dependent. I used a correlation regression model which takes the correlated observations into account when it estimates sampling variation such as regression-coefficient standard errors. This tends to provide larger standard errors in order to adjust for multiple observations derived from the same patient. Consequently, p-values of the predictor variables in the model are also larger.(286) The model was fitted using a backward stepwise selection procedure. In the first step all variables that were significant at the p=0.05 level in the univariate analysis were entered into the multivariate model. In the second step, variables not statistically or clinically significant with the vaccine uptake were eliminated from the model. Observations from asthma patients (13,368/194,314) with a death record were removed from the analysis. The level of statistical significance was set at p=0.05 and all statistical tests were two-sided. The analyses were performed using RStudio (Version 1.0.143).(252)

6.3 Results

Primary care cohort dataset

In total, 614,182 (33.6%) influenza vaccinations were administered during 1,830,772 person-seasons of observation across 16 influenza seasons. Overall high influenza vaccine uptake was observed for females (38.7%), school-aged (5-11 years old) children (24.5%) and older adults (≥75 years old) (82.8%), people with a higher socioeconomic status (33.9%), people living in remote rural areas (41.6%), people with COPD (75.6%), chronic heart disease (76.3%), chronic liver disease (56.7%), chronic neurological disease (75.2%), diabetes (76.7%), immunosuppression (61.2%), people with >5 medical conditions (83.2%), people with an influenza (80.5%) and pneumococcal (79.6%) vaccination history in the previous season, in ex-smokers (63.7%), people with a Charlson score 5 (78.0%), people with >5 primary care consultations for acute respiratory illness in the previous season (70.4%) and people with 4 emergency hospital admissions in the previous season (60.0%).(see Table 6.5)

After adjustment, the OR for vaccine uptake was high in females, adults aged >_65 years old, people living in remote rural areas, people with chronic conditions (COPD, chronic heart disease, chronic liver disease, chronic neurological disease, diabetes and immunosuppression), people with a previous influenza or pneumococcal vaccination, ex-smokers, people with five primary care consultations in the previous season and people with two emergency admissions in the previous season.(see Table 6.6) No differences in adjusted ORs for vaccine uptake was found amongst people in different socioeconomic status.(see Table 6.6)

Table 6.5: Vaccine status by demographic and other clinical characteristics for 194,314 asthma patients (1,830,772 patient-seasons), Scotland, 2000/01 to 2016/17

Characteristic	Vaccinated (% total) (n= 614,182 seasons)	Unvaccinated (% total) (n= 1,216,590 seasons)	Vaccine Uptake (%)	Vaccine uptake unadjusted OR (95% CI)
Gender				
Female (reference)	59.5	47.5	38.7	NA
Male	40.5	52.5	28.0	0.615 (0.61 to 0.62)
Age group (years)				
0-1	0.01	0.1	10.1	0.023 (0.020 to 0.030)
2-4	0.6	1.0	24.1	0.066 (0.060 to 0.070)
5-11	5.3	8.2	24.5	0.067 (0.066 to 0.070)
12-17	4.9	12.2	17.0	0.043 (0.040 to 0.044)
18-44	24.9	56.1	18.3	0.046 (0.045 to 0.050)
45-64	32.6	18.8	46.7	0.180 (0.178 to 0.185)
65-74	17.8	2.2	80.2	0.840 (0.820 to 0.860)
≥75 (reference)	13.9	1.5	82.8	NA
Deprivation quintile				
1[a] (reference)	18.1	17.9	33.7	NA
2	21.5	21.0	34.1	1.02 (1.01 to 1.03)
3	19.5	19.4	33.6	1.00 (0.99 to 1.01)
4	20.5	20.2	33.9	1.01 (1.00 to 1.02)
5	18.9	19.5	32.9	0.96 (0.95 to 0.97)
Urban/rural score				
1 (reference)	35.4	37.5	32.3	NA
2	33.4	34.2	33.0	1.03 (1.02 to 1.04)
3	9.2	8.6	34.9	1.12 (1.11 to 1.14)
4	2.3	1.9	37.9	1.28 (1.25 to 1.31)
5	1.5	1.4	35.1	1.13 (1.10 to 1.16)
6	10.8	10.3	34.7	1.11 (1.10 to 1.13)
7	2.9	2.3	39.4	1.36 (1.34 to 1.39)
8[b]	3.6	2.5	41.6	1.50 (1.47 to 1.52)
COPD	7.2	1.2	75.7	6.56 (6.43 to 6.69)
Chronic heart disease	11.1	1.8	76.3	7.04 (6.93 to 7.15)
Chronic liver disease	0.6	0.2	56.7	2.60 (2.48 to 2.73)
Chronic neurological disease	4.4	0.7	75.2	6.23 (6.08 to 6.38)
Diabetes	8.8	1.4	76.7	7.07 (6.94 to 7.19)
Immunosuppression	0.5	0.2	61.2	3.14 (2.97 to 3.32)
Number of risk groups				
1 (reference)	70.9	93.4	27.7	NA
2	17.2	5.0	63.6	4.56 (4.51 to 4.61)
3	8.2	1.2	77.0	8.72 (8.56 to 8.88)
4	2.9	0.3	81.1	11.18 (10.81 to 11.57)
5	0.8	0.1	81.2	11.25 (10.54 to 12.01)

>5	0.1	0.01	83.2	12.95 (10.72 to 15.63)
Influenza vaccine in the previous season				
Yes	76.1	9.3	80.5	30.88 (30.62 to 31.14)
No (reference)	23.9	90.7	11.8	NA
Pneumococcal vaccine				
Yes	4.2	0.6	79.6	8.05 (7.83 to 8.27)
No (reference)	95.8	99.5	32.7	NA
Smoking status				
Non-smoker (reference)	30.6	16.8	47.9	NA
Ex-smoker	18.9	5.5	63.7	1.90 (1.88 to 1.94)
Current smoker	13.6	9.8	41.2	0.76 (0.75 to 0.77)
Not recorded	36.9	68.0	21.5	0.30 (0.295 to 0.31)
Charlson score				
0 (reference)	7.5	7.6	33.2	NA
1	14.4	6.0	55.0	2.46 (2.42 to 2.49)
2	4.9	0.9	73.6	5.61 (5.47 to 5.75)
3	2.0	0.3	76.6	6.58 (6.33 to 6.84)
4	0.8	0.1	77.4	6.89 (6.48 to 7.31)
5	0.4	0.1	78.0	7.11 (6.50 to 7.78)
>5	0.7	0.2	67.5	4.18 (3.96 to 4.41)
Primary care consultations for acute respiratory illness				
0 (reference)	82.0	87.7	32.1	NA
1	10.6	5.8	48.1	1.96 (1.94 to 1.98)
2	2.8	1.1	56.7	2.78 (2.71 to 2.84)
3	1.1	0.3	60.9	3.30 (3.18 to 3.43)
4	0.5	0.1	63.7	3.72 (3.50 to 3.95)
5	0.3	0.1	68.6	4.63 (4.24 to 5.06)
>5	0.4	0.1	70.4	5.05 (4.67 to 5.45)
Emergency hospital consultations				
0 (reference)	89.3	91.5	33.0	NA
1	6.3	3.0	51.8	2.18 (2.15 to 2.21)
2	1.3	0.5	58.0	2.80 (2.70 to 2.90)
3	0.4	0.1	58.5	3.04 (2.85 to 3.23)
4	0.2	0.1	60.0	3.04 (2.76 to 3.35)
5	0.1	0.03	58.5	2.86 (2.47 to 3.32)
>5	0.1	0.04	52.8	2.27 (2.01 to 2.56)

Abbreviations: OR: odds ratio; CI: confidence interval; NA: not applicable;
a Most socioeconomically deprived
b Remote rural areas
Missing data (available data): Deprivation quintile 2.0% (1,796,253 patient-seasons);
Urban/rural score 1.0% (1,808,751 patient-seasons);
Charlson score 79.7% (372,403 patient-seasons);
Primary care consultations for acute respiratory illness 4.0% (1,756,516 patient-season);
Emergency hospital admissions 4.0% (1,756,516 patient-season)

Table 6.6: Vaccine status by demographic and other clinical characteristics for 180,951 asthma patients (1,732,799 patient-seasons), Scotland, 2000/01 to 2016/17

Characteristic	Adjusted OR	Adjusted 95% CI
Gender		
Female (reference)	NA	NA
Male	0.79	0.78 to 0.80
Age group (years)		
0-1	0.21	0.16 to 0.28
2-4	0.36	0.34 to 0.38
5-11	0.32	0.31 to 0.33
12-17	0.18	0.175 to 0.19
18-44	0.19	0.18 to 0.20
45-64	0.41	0.39 to 0.43
65-74	1.04	1.00 to 1.08
≥75 (reference)	NA	NA
Deprivation quintile		
1[a]	NA	NA
2	1.00	0.98 to 1.02
3	0.98	0.96 to 1.00
4	0.97	0.95 to 0.99
5	0.98	0.96 to 1.00
Urban/rural score		
1	NA	NA
2	0.96	0.95 to 0.98
3	0.97	0.95 to 0.99
4	1.08	1.03 to 1.12
5	0.97	0.92 to 1.02
6	0.98	0.95 to 1.00
7	1.08	1.04 to 1.13
8[b]	1.12	1.08 to 1.16
COPD	1.21	1.15 to 1.26
Chronic heart disease	1.48	1.42 to 1.54
Chronic liver disease	1.41	1.28 to 1.55
Chronic neurological disease	1.22	1.15 to 1.29
Diabetes	1.80	1.73 to 1.87
Immunosupression	1.75	1.55 to 1.97
Influenza vaccine in previous season		
Yes	17.81	17.57 to 18.06
No	NA	NA
Pneumococcal vaccine in previous season		
Yes	1.20	1.16 to 1.25
No	NA	NA
Smoking status		
Non-smoker	NA	NA
Ex-smoker	1.06	1.04 to 1.08
Current smoker	0.86	0.84 to 0.87
Not recorded	0.55	0.547 to 0.56
Primary care consultations for acute respiratory illness in previous season		
0	NA	NA
1	1.53	1.50 to 1.55
2	1.73	1.66 to 1.79

3	1.84	1.73 to 1.96
4	1.93	1.75 to 2.13
5	2.30	1.97 to 2.66
>5	2.14	1.88 to 2.45
Emergency hospital consultations in previous season		
0	NA	NA
1	1.31	1.27 to 1.34
2	1.41	1.32 to 1.51
3	1.30	1.14 to 1.47
4	1.14	0.92 to 1.42
5	1.31	0.94 to 1.82
>5	1.24	0.94 to 1.64

Abbreviations: OR: odds ratio; CI: confidence interval; NA: not applicable;
a Most socioeconomically deprived
b Remote rural areas
Missing data (available data): Gender 0.01% (1,732,792); Deprivation quintile 1.84% (1,700,951 patient-seasons); Urban/rural score 1.15% (1,712,934 patient-seasons); Primary care consultations for acute respiratory illness 3.80% (1,666,931 patient-season); Emergency hospital admissions 3.80% (1,666,931 patient-season);

Emergency hospital admission dataset

There were 8,231 emergency hospital admissions due to influenza or pneumonia out of 6,232 patients with an asthma diagnosis in the community over 16 influenza seasons. The vaccine uptake out of the total admissions was 65.9% (5,422/8,231). The coverage in females was higher but not significantly different from males. Higher uptake was observed in older age groups with those $\geq$ 75 years old having an uptake of 31.0%.

The coverage was higher from admissions with low socioeconomic status. Higher uptakes were observed from large urban areas; however, the differences were not statistically significant. Admissions from people chronic medical conditions were more likely to be vaccinated. Highest uptake was detected amongst admissions were COPD as a comorbidity. The uptake was higher in admissions where patients had more than one at-risk condition. The receipt of an influenza vaccine in the previous season increased the propensity of being vaccinated, while having a pneumococcal vaccine in the past decreased the propensity of being vaccinated against influenza. Non-smokers and ex-smokers were more likely to have been vaccinated than current smokers. Charlson score showed a decrease in vaccine uptake as the number of at-risk conditions increased. Significantly higher coverage was observed only in those with two or three primary care consultations for acute respiratory illness in the previous season. Highest uptake was found in those with no previous consultations however the number of admissions in the unvaccinated when compared to the vaccinated. Similarly, the highest uptake was observed in those with no previous season emergency admission but most admissions came from unvaccinated individuals. Significant higher uptake levels were observed in those with one or more emergency admissions in the previous year, except in those with three admissions with insignificant difference.(see Table 6.7).

Table 6.7: Baseline characteristics by vaccine status of emergency admissions due to influenza or pneumonia, Scotland, 2000/01 to 2016/17 (8,231 admissions)

Characteristic	Vaccinated (% total) (n=5,422 admissions)	Unvaccinated (% total) (n=2,809 admissions)	Vaccine Uptake (%)	Vaccine uptake adjusted OR (95% CI)
Gender				
Female (reference)	3,043 (56.1)	1,619 (57.6)	37.0	NA
Male	2,379 (43.9)	1,190 (42.4)	28.9	1.06 (0.97 to 1.17)
Age group (years)				
0-1	5 (0.1)	5 (0.2)	0.1	0.27 (0.08 to 0.92)
2-4	26 (0.5)	79 (2.8)	0.3	0.09 (0.06 to 0.14)
5-11	69 (1.3)	130 (4.6)	0.8	0.14 (0.10 to 0.19)
12-17	46 (0.9)	74 (2.6)	0.6	0.17 (0.11 to 0.24)
18-44	362 (6.7)	730 (26.0)	4.4	0.13 (0.11 to 0.15)
45-64	1,149 (21.2)	760 (27.1)	14.0	0.40 (0.36 to 0.46)
65-74	1,211 (22.3)	350 (12.5)	14.7	0.92 (0.80 to 1.07)
≥75 (reference)	2,554 (47.1)	681 (24.2)	31.0	NA
Deprivation quintile				
1[a]	1,209 (22.3)	753 (26.8)	15.0	NA
2	1,419 (26.2)	674 (24.0)	17.0	1.31 (1.15 to 1.49)
3	974 (18.0)	480 (17.1)	12.0	1.26 (1.10 to 1.46)
4	971 (17.9)	444 (15.8)	12.0	1.36 (1.18 to 1.57)
5	743 (13.7)	364 (13.0)	9.0	1.27 (1.09 to 1.48)
Urban/rural score				
1	2,198 (40.5)	1,115 (39.7)	27.0	NA
2	1,749 (32.3)	958 (34.1)	21.1	0.93 (0.83 to 1.03)
3	458 (8.5)	228 (8.1)	5.6	1.02 (0.86 to 1.21)
4	107 (2.0)	57 (2.0)	1.3	0.95 (0.69 to 1.32)
5	83 (1.5)	45 (1.6)	1.0	0.94 (0.65 to 1.36)
6	521 (9.6)	223 (7.9)	6.3	1.19 (1.00 to 1.41)
7	101 (1.9)	39 (1.4)	1.2	1.31 (0.90 to 1.91)
8[b]	133 (2.5)	62 (2.2)	1.6	1.09 (0.80 to 1.48)
COPD	1,707 (31.5)	552 (19.7)	20.7	1.88 (1.68 to 2.10)
Chronic heart disease	1,684 (31.1)	453 (16.1)	20.5	2.34 (2.09 to 2.63)
Chronic liver disease	113 (2.1)	66 (2.4)	1.4	0.89 (0.65 to 1.20)
Chronic neurological disease	778 (14.4)	259 (9.2)	9.5	1.65 (1.42 to 1.91)
Diabetes	1,001 (18.5)	272 (9.7)	12.2	2.11 (1.83 to 2.44)
Immunosuppression	111 (2.1)	63 (2.2)	1.4	0.91 (0.67 to 1.25)
Number of risk groups				
1	1,550 (28.6)	1,542 (54.9)	18.8	NA
2	1,343 (24.8)	497 (17.7)	16.3	2.69 (2.37 to 3.05)
3	1,436 (26.5)	461 (16.4)	17.5	3.10 (2.73 to 3.52)
4	804 (14.8)	206 (7.3)	9.8	3.88 (3.28 to 4.60)
5	230 (4.2)	89 (3.2)	2.8	2.57 (1.99 to 3.32)

>5	59 (1.1)	14 (0.5)	0.7	4.19 (2.33 to 7.54)
Influenza vaccine in the previous season				
Yes	4,677 (86.3)	831 (29.6)	56.8	14.94 (13.36 to 16.71)
No	745 (13.7)	1,978 (70.4)	9.1	NA
Pneumococcal vaccine				
Yes	269 (5.0)	26 (0.9)	3.3	5.59 (3.73 to 8.38)
No	5,153 (95.0)	2,783 (99.1)	62.6	NA
Smoking status				
Non-smoker	1,051 (19.4)	350 (12.5)	12.8	NA
Ex-smoker	1,798 (33.2)	375 (13.4)	21.8	0.58 (0.49 to 0.68)
Current smoker	1,012 (18.7)	583 (20.8)	12.3	1.60 (1.36 to 1.88)
Not recorded	1,561 (28.8)	1,501 (53.4)	19.0	0.35 (0.30 to 0.40)
Charlson score				
0	356 (6.6)	253 (9.0)	4.3	NA
1	1,386 (25.6)	629 (22.4)	16.8	1.57 (1.30 to 1.89)
2	856 (15.8)	277 (9.9)	10.4	2.20 (1.78 to 2.71)
3	565 (10.4)	177 (6.3)	6.9	2.27 (1.80 to 2.87)
4	309 (5.7)	112 (4.0)	3.8	1.96 (1.50 to 2.57)
5	187 (3.5)	57 (2.0)	2.3	2.33 (1.66 to 3.27)
>5	238 (4.4)	102 (3.6)	2.9	1.66 (1.25 to 2.20)
Primary care consultations for acute respiratory illness in previous year				
0	4,276 (78.9)	2,252 (80.1)	52.0	NA
1	583 (10.8)	276 (9.8)	7.1	1.11 (0.96 to 1.30)
2	205 (3.8)	78 (2.8)	2.5	1.38 (1.06 to 1.81)
3	110 (2.0)	31 (1.1)	1.3	1.87 (1.25 to 2.79)
4	45 (0.8)	26 (0.9)	0.5	0.91 (0.56 to 1.48)
5	38 (0.7)	11 (0.4)	0.5	1.82 (0.93 to 3.57)
>5	54 (1.0)	24 (0.9)	0.7	1.18 (0.73 to 1.92)
Emergency hospital consultations in previous year				
0	3,254 (60.0)	1,834 (65.3)	39.5	NA
1	1,112 (20.5)	428 (15.2)	13.5	1.46 (1.29 to 1.66)
2	478 (8.8)	206 (7.3)	5.8	1.31 (1.10 to 1.55)
3	197 (3.6)	99 (3.5)	2.4	1.12 (0.88 to 1.44)
4	128 (2.4)	41 (1.5)	1.6	1.76 (1.23 to 2.51)
5	56 (1.0)	17 (0.6)	0.7	1.86 (1.08 to 3.20)
>5	86 (1.6)	72 (2.6)	1.0	0.67 (0.49 to 0.93)

Abbreviations: OR: odds ratio; CI: confidence interval; NA: not applicable

a Most socioeconomically deprived

b Remote rural areas

Missing data (available data): Deprivation quintile 2.4% (8,031 admissions);
Urban/rural score 1.9% (8,077 admissions);
Charlson score 33.1% (5,504 admissions);
Primary care consultations for acute respiratory illness 2.7% (8,008 admissions);
Emergency hospital consultations in previous year 2.7% (8,008 admissions)

Primary care prescriptions dataset

GPs prescribed 8,006,171 medications in 134,941 patients with asthma over 15 influenza seasons (2002/03 to 2016/17). The number of prescribed medications increased from 173,449 in 2002/03 to 652,585 in 2016/17 (see Appendix A15; p 330). There were 57.1% of asthma-related medications (e.g. bronchodilators, inhaled and oral corticosteroids) issued out of the total number of prescriptions (see Appendix A16; p 330). The number of prescriptions in each BTS step developed in this study was also calculated. The highest number of prescriptions was observed in Step 1 (35.3%) while Step 2 included the least prescriptions (14.5%) (see Appendix A17; p 330). The low vaccine uptake in Step 2 could be due to patients with medium or high dose of ICS being assigned to Step 3 rather than to Step 2 when they also had OCS prescriptions during a season.

The vaccine status was identified for 7,271,777 out of the 8,006,171 prescriptions based on the primary care cohort. Out of the total 7,271,772 prescriptions 65.2% (4,742,979) derived from asthma patients with an influenza vaccination. The highest vaccine uptake was observed in Step 1, which included lower doses of ICS for children and adults with asthma. The lowest uptake was found in patients with prescribed medications other than inhaled or oral steroids (see Table 6.8).

Table 6.8: Number of prescriptions in each of the four BTS steps by vaccine status (n=7,271,777)

BTS step	Vaccinated (% total) (n=4,742,979 prescriptions)	Unvaccinated (% total) (2,528,798 prescriptions)	Vaccine uptake (%)	Vaccine uptake adjusted OR (95% CI)
Step 0	638,860 (13.5)	733,019 (29.0)	8.8	0.317 (0.315 to 0.318)
Step 1	1,844,842 (38.9)	977,985 (38.7)	25.4	0.685 (0.683 to 0.688)
Step 2	851,879 (18.0)	306,414 (12.1)	11.7	1.01 (1.00 to 1.02)
Step 3	1,407,398 (29.7)	511,380 (20.2)	19.4	NA

Abbreviations: BTS: British Thoracic Society; OR: odds ratio; CI: confidence interval
Step 0: no other step;
Step 1: no medium or high dose of ICS;
Step 2: medium or high dose of ICS;
Step 3: step 2 & OCS or OCS only or OCS & step 0 or OCS & step1
Missing values: no vaccine status for 734,394 prescriptions (9.2%)

6.4 Discussion

Summary of main findings

This is the first analysis to explore demographic and clinical characteristics of influenza vaccine groups in individuals with asthma across multiple influenza seasons in Scotland. In people with asthma the overall vaccine coverage was below the desirable level. Females, adults aged ≥65 years old, individuals living in remote rural areas, with comorbidities, with history of influenza or pneumococcal vaccination, ex-smokers and with history of five primary care visits and two emergency hospital admissions were more likely to have been vaccinated in the current season. Individuals with prescribed medications were also more likely to have been vaccinated. Particularly, asthma-related prescriptions including inhaled or oral steroids were associated with vaccination.

Comparisons with existing literature

In this analysis, females were more likely to be vaccinated than males, a finding also observed in previous studies.(287)(288) The uptake pattern differed between children and adults. Specifically, the higher coverage found in younger children and in older adults, confirmed previous findings.(104)(289)(290) However, only adults over 65 years old were significantly more likely to be vaccinated compared to younger age groups. This pattern could be explained by the recommendation that older adults (especially over 65 years old) are vaccinated irrespective of other risk factors such as asthma.

Individuals with lower socioeconomic status who were admitted to hospital were more likely to have been vaccinated. Previous studies have also shown that lower socioeconomic status was associated with higher chance of vaccination.(291-294) There is, however, conflicting evidence, particularly for children. Specifically, others have found that vaccine uptake was lower in children of parents with lower income or with less than a high school education.(295-297) However, the adjusted vaccine uptake ORs in this study showed no difference for the primary care population. No substantial differences were observed in vaccine uptake between individuals living in large urban and small remote areas. The presence of asthma co-morbidities increased the uptake of the vaccine. People with additional chronic conditions tend to have more healthcare

visits which, by extension, increases the probability of being recommended annual vaccination and receiving one opportunistically during a health care visit for another reason.(288)(291)(298) The receipt of influenza or pneumococcal vaccination in the previous year was strongly related with current year vaccination. A person with asthma who is regularly vaccinated may be more likely to continue vaccination potentially reflecting their personal belief in the importance of the vaccination or strong physician recommendation due to the severity of their underlying condition. This has also been shown in the case of the 2009 H1N1 pandemic where the receipt of the seasonal vaccine was a significant predictor of the pandemic vaccine.(298)(299) Thus, individuals that tend to get vaccinated may be more likely to accept additional vaccinations even in the same year for the same infection.(298)(299) This study found that current smokers were less likely to receive vaccination compared to non- or ex-smokers. Previous studies have shown the association of no vaccine receipt in current smokers not only in asthma, but also in other chronic respiratory conditions.(186)(289)(292) This is an important finding as the combination of smoking and no vaccine protection against influenza puts people with asthma or other respiratory conditions at an increased risk of respiratory exacerbations. Patients with primary care or hospital care related visits in the previous season had a higher possibility to be vaccinated. A reverse pattern of vaccination was seen between primary and secondary healthcare visits. The increase of primary care consultations was associated with a vaccine uptake increase while no difference in vaccination was found in individuals with four or more hospital admissions. Regular primary healthcare visits could reflect a person's healthy lifestyle or recognition of the need for protection by the healthcare providers and patients resulting in increased likelihood of vaccination.(300)(301) In this analysis, vaccinated individuals with asthma were more likely to have received a prescribed medication. The prescription of preventive asthma-related medications such as bronchodilators and inhaled corticosteroids was particularly strongly associated with vaccine uptake. Other studies have hypothesised that the use of asthma medications reflects more severe asthma and increased number of healthcare encounters.(288)(300) Thus this could be associated with higher chance of vaccine recommendation and administration.

Strengths and limitations

The main strength of this analysis is the use of a large primary care dataset including 194,319 people with asthma in over a decade of observational data. Thus, the external validity of this analysis is probably high, and the study's findings are likely generalisable to the asthma population in Scotland. Furthermore, the inclusion of different patient characteristics (e.g. demographics, socio-economic status and clinical information) allowed for an exploration of differences of vaccine uptake between groups. However, there are several limitations in this analysis. First, unmeasured confounding could still have biased the vaccine uptake estimates in this analysis. Second, some individuals' characteristics were less completely ascertained due to insufficient data in early seasons (e.g. Charlson score) or no regular recording of these type of information (e.g. smoking status) by the GPs. Finally, the asthma severity status was not available to this analysis. This was due to insufficient data on asthma symptoms, pulmonary function tests and the use of GP asthma prescriptions where doses and other related information are recorded as free-text and could not be accurately determined for all patients.

Implications and conclusion

In Scotland, the influenza vaccine is given free of charge to all patients with asthma who are on continuous or repeated inhaled or oral corticosteroids or have a history of hospital admission due to asthma attack in the last 12 months. This study also found higher vaccine uptake in people with asthma prescriptions and a history of emergency admission in the last season. However, overall uptake rates based on annual national reports and also confirmed in this analysis remain suboptimal. On the other hand, age-based immunisation strategies seem to be more effective than at-risk strategies based on the increased or desirable uptake levels in older adults ($\geq$65 years old) found in this study.(302).Healthcare providers should also be more informed regarding the latest evidence on VE, safety and burden of the infection in the asthma population.(104) Thus, providers are more likely to recommend the vaccine based on clear and timely evidence. Finally, healthcare providers should promote influenza vaccination particularly in males, younger age groups, active smokers and those with no vaccination history for influenza as they are less likely to be vaccinated. In conclusion,

more work is needed to improve the current low vaccination rates in the Scottish asthma population. Specifically, vaccination strategies should aim to improve the coverage in males, in younger age groups, in smokers and in those without a previous history of vaccination. A qualitative study could be performed to explore the barriers and motivators for vaccination between males and females, in younger adults, in smokers and in those without regular vaccination histories for influenza. The focus on these subgroups would probably increase the overall uptake in the asthma population since these subgroups are less likely to follow the influenza vaccine recommendations. Thus, the gap between vaccination policy and implementation in the clinical settings is likely to be bridged.

6.5 Summary to Chapter 6

In Scotland, the influenza vaccine coverage in the asthma population is below the 75% WHO recommended target. Individuals with characteristics including males, children and young adults, current smokers, no history of previous vaccination, no regular health visits, not on asthma medications (e.g. inhaled or oral steroids) were more often found in the unvaccinated group. Uptake rates could be increased if the vaccination programme focuses on these groups of the asthma population. Thus, this will improve the implementation of the current vaccination guidelines at the clinical practice.

Findings from this analysis and from studies in previous chapters will now be discussed in the following chapter. Specifically, all findings in this thesis will be discussed in terms of strengths and weaknesses and compared with other literature. Finally, the chapter will aim to identify all implications and conclusions resulting from this thesis that are applicable to vaccination policy, research community, healthcare practice and the greater public.

Chapter 7. General discussion

The aim of this final chapter is to provide an overall description, evaluation and context of my research findings in relation to current literature. Recommendations and future work arising from this thesis will also be provided. Specifically, in this chapter, I will summarise the findings from my research work in Chapters 3, 5 and 6. Strengths and weaknesses from the aforementioned chapters will also be discussed. Then I will reflect and integrate the findings of this thesis in relation to existing research evidence. Implications to policy, research and practice as a result of this thesis will also be considered. Finally, I will provide all conclusions that can be derived from this thesis. More detailed discussions for the research work undertaken in this thesis have been already provided in previous individual chapters.

7.1 Summary of main findings

The systematic review (Chapter 3) showed that influenza vaccines offered protection for people with asthma from influenza infection and other influenza induced complications. An overall VE of 45.0% was observed against laboratory-confirmed influenza infection. Protection against asthma related hospitalisation was also found which ranged from 59.0% to 78.0%. Finally, vaccines were safe and well tolerable in people with asthma.

The TND case-control study (Chapter 5) found an overall VE of 48% against influenza infection in children and adults with asthma in Scotland. Significant positive VE was also seen in years with good antigenic match between the circulating and vaccine strains and for particular strains such as A(H1N1) and B. In addition, vaccination offered protection for all circulating influenza strains in adults less than 55 years old. While the LAIV offered substantial protection against influenza B in children with asthma.

The univariate analysis on vaccine uptake (Chapter 6) found characteristics of people with asthma which increase the likelihood of vaccination. Specifically, individual characteristics including females, younger children and older adults, co-morbidity, vaccination history, recent healthcare visit history and asthma prescription were related with higher influenza vaccine uptake.

7.2 Strengths of the research program

This research work added new evidence regarding the protective effects of the seasonal influenza vaccination in the asthma population. The uniqueness of this PhD thesis lies upon the explicit focus on the asthma population. The main strength of this work derives from the conduct of a large review of the literature and two large primary studies which enabled the exploration of research questions on vaccine effectiveness and safety in children and adults with asthma. Lack of substantial immunological, clinical and research personal experience on this topic was addressed by the independent journal peer-review of my systematic review work, carrying out my primary work analyses within the SIVE II project dataset and the overall support from the SIVE II project team and the AUKCAR.

Specifically, I used a systematic review to synthesise any available evidence of the protective and adverse events of seasonal influenza vaccination in asthma. The use of a systematic review instead of a narrative review aimed to reduce any bias during the review process by using pre-specified systematic and reliable methods. My review findings were also statistically synthesised (e.g. via meta-analyses), increasing the strength of evidence by providing pooled estimates on vaccine protection. In addition, the journal publication (BMJ Open) and the registration in PROSPERO (CRD42016037219)(303) of my systematic review protocol standardised and guided the process of systematic review.(146) Thus, any deviations between my predetermined systematic review protocol and the completed systematic review were kept to a minimum by reducing any potential reviewer bias which can happen when the knowledge of the systematic review findings shapes the review process. The completed review also underwent an independent journal peer-review (Clinical Infectious Diseases) which improved the interpretation and presentation of my work.(234) As a first-time author of a systematic review, I also built a large and multidisciplinary review team who guided and made me aware of any errors during the review process.

This PhD work program also adds new valuable information on the preventive capacity of the seasonal influenza vaccine in children and adults with asthma against laboratory confirmed influenza. An exploration of the vaccine uptake patterns in the asthma

population was also carried out in this PhD, evidence from which could help to tackle the sub-optimal coverage rate seen in the asthma population at national level by targeting patients with asthma not receiving the vaccination. My main primary work was part of the wider SIVE II project which aims to assess the effectiveness and safety of the influenza vaccination amongst at-risk groups.(128) The SIVE II project was led by an expert team (an led by my supervisor Prof. Colin Simpson), with a record of previous published work on the area of influenza vaccines and asthma research. The SIVE II project also determined all its epidemiological methods and statistical analyses in their protocol published in BMJOpen, which I contributed to.(128) My statistical analyses were guided and assessed by the SIVE II statistical team ensuring the soundness of my work and the subsequent outputs. The large sample size of my case-control study and univariate analysis enabled me to explore additional research questions not pre-determined in the SIVE II project.

The main strength of the TND case-control study in this thesis was that it used data from the national GP Scottish routine sentinel surveillance program which reduced concerns regarding the validity of the VE estimates produced (although I still carried out validation work comparing sentinel GP vs. non-GP sentinel results).(304) In addition, cases and controls were more comparable since both groups were retrieved from the same population (patients with ILI seeking care) - a major advantage of the nested TND case-control study over traditional case-control studies. Another strength of this study design lies in the fact that all data on vaccine exposure and other baseline characteristics were determined before the allocation of the patients into cases or controls. Thus, this should have minimised any recall bias from the asthma patients or observer bias derived from the research investigators.

7.3 Limitations of the research program

There were also several limitations related to the methodology and the datasets that were used in this research work.

Pitfalls with the evidence search and synthesis in the literature review

During my systematic review many studies assessed the effectiveness of the vaccines in individuals with chronic respiratory diseases and not explicitly in asthma. Thus, I

had to contact many authors to identify any separate analyses on the asthma population. However, some authors either did not reply to my requests for collaboration, or I was not able to find any contact details. In addition, some authors did not have access to their data any longer and could not confirm to me any analyses in the asthma subgroup. Thus, it is possible my systematic review failed to include some eligible studies. Another issue regarding my systematic review is availability of agreed rigorous criteria for the provision of pooled estimates deriving from epidemiological or clinical heterogeneous (e.g. participants, interventions, outcome measures and study designs) studies. As a result, I pooled results from two studies in each of my three meta-analyses as they were homogenous in terms of study design, intervention and outcome measure. However, this approach prohibited meta-analyses using results from more than two studies. Thus, the estimated effect size and the precision of the VE estimates in this review can be still questioned. According to previous work by Beyer et al protective effects of the influenza vaccines can be hidden when data from original studies are meta-analysed based on multiple criteria (e.g. vaccine types, study designs, populations and type of outcomes, virus circulation and vaccine mismatch) that are not related to the vaccine.(305)

Confounding and bias in the primary research work

The TND is now the most widely used study design used to answer the question of VE against confirmed influenza infection. However, we need to bear in mind that this is still an observational, analytic study design which cannot directly determine causation between vaccine exposure and influenza prevention. Thus, the internal validity of the TND study may still be questioned. Measured and unmeasured confounding could still have affected the VE estimates in this study given that their effect on the magnitude and direction of the VE estimates were not determined. In my TND study, swab samples were also taken from hospital settings. Thus, there could be individuals with more severe influenza-related outcomes e.g. hospitalisations due to influenza. However, if a large number of individuals are hospitalised and at a second time swabbed for influenza when the virus was no more detectable this could have led to false negative results.(231) Viral interference is another potential issue that could have undermined the validity of the TND, and happens when transient non-specific immune

protection against acute respiratory illness occur after a previous exposure to a respiratory infection. According to Foppa et al, children who received the inactivated trivalent vaccine had higher risk of having a non-influenza illness compared to the placebo group.(231) If this pattern between the vaccine and the likelihood of a non-influenza illness indeed exists could threaten the validity of the TND resulting to overestimation of the true VE when it comes to impact on all ILI (rather than true influenza alone).

In the TND study, I measured the effectiveness of the vaccine in preventing confirmed influenza illness. Nonetheless, the vaccine could also modify the expression of the influenza symptoms when an immunised individual is infected with the virus. This phenomenon could bias the ORs from the TND study as the ORs would not only represent the risk of infection but could also represent the risk of having more severe symptoms compared to an unvaccinated individual. However, it remains unclear how the modification of influenza symptoms followed by the vaccination will influence the VE estimates.(231) A potential confounder that can also affect the VE estimates of the TND study is the rate that the individuals in my study came into contact with other influenza infected individuals.(230) For example, female patients were more likely to get vaccinated in the TND and the univariate analysis, but at the same time mothers of young children are more likely to be in contact with children who are more likely to get infected (see section 1.3.1 in Chapter one). It was however difficult to find a relevant variable that could assess the existence of such a confounding effect.

Bias could also be introduced in the TND study if the influenza vaccination had indirect effects, other than preventing the influenza virus. According to Lipsitch et al, if this is the case, the direct protective effect of the vaccine on the influenza could have been imitated or hidden by those indirect effects leading to under- or over-estimation of the VE estimates.(306) The first potential bias could occur if the vaccine has a protective effect on non-influenza ILI. This potential bias mechanism does not fit with claim by the Foppa et al regarding the higher risk of non-influenza ILI among vaccinated children.(231) This potential bias means that the vaccine might also decrease the likelihood of an individual being infected with a non-influenza virus compared to the general population. Thus, individuals that are swabbed for ILI

symptoms are more likely to be tested positive for influenza or less likely to be tested negative for influenza than an individual in the general population. If this had occurred in my study, it could have biased and underestimated the VE estimates since some of the direct protective effect of the vaccine could have been masked from the beneficial effect against other non-influenza ILI.(306) A second potential bias could have occurred if the influenza infection induced a non-specific immune protection against non-influenza illnesses.(306) Lipsitch et al hypothesised that in this scenario the influenza vaccine will increase the risk of a non-influenza ILI and the chance of being tested negative for influenza infection.(306) Consequently, the VE estimates will be overestimated since vaccinated individuals will be more likely to be controls than in the general population.(306) The final potential bias could result from the fact that individuals at higher risk for influenza infection are more likely to contract the infection earlier in the season and be protected for a second infection during that season. Similarly, higher risk individuals will be vaccinated more rapidly during the influenza season. However, the presence of within-season decline in immune protection provided by either past infections or vaccination will eventually decrease the VE estimates over the course of the season.(306)

The main widely accepted methodological advantage of the TND study over the traditional case-control study is the exclusion of bias derived from confounding by healthcare seeking behaviour.(239) This behaviour refers to the likelihood of an individual seeking care when in the presence of respiratory infection symptoms. Thus, the healthcare seeking behaviour is treated by the TND studies as a binary variable whereby an individual will either seek or not seek care. However, the propensity of seeking care is dynamic and changes constantly based on other behaviours (e.g. hand washing) or characteristics such as age or high-risk status. Therefore, it is an oversimplification to adjust for healthcare seeking behaviour by using a binary indicator and it will still remain partially unobserved. Consequently, according to Sullivan et al the TND study will not completely account for the effects of this confounder.(239)

Another limitation is the use of data from medical records for the ascertainment of exposure, outcome and baseline characteristics. The accuracy of these data is higher

than in studies based on self-report and introducing subsequently recall bias.(239) However, misclassification bias in study variables is still possible particularly given the retrospective nature of the primary studies in this thesis. Nonetheless, any errors in the medical records are likely to be random and thus have a smaller effect on VE estimates. Another limitation is related to the definition of the influenza-like illness symptoms, for which there are widely agreed criteria in use in Scotland. Therefore, GPs or other healthcare providers may have been more likely to take swab samples from people with at-risk conditions such as asthma. In my TND study, I found that in patients with asthma, the odds of being swabbed for influenza were 1.76 (95% CI: 1.60 to 1.94) higher than in non-asthma patients (see Appendix A18; p 331). However, the odds of being infected with influenza were also higher and up to 1.28 (95% CI: 1.17 to 1.41) in patients with asthma compared to non-asthma patients (see Appendix A19; p 332).

A central assumption of the TND that there is only direct protection afforded by the vaccine may be violated in the following scenario. Vaccines that alter viral shedding may reduce transmission of infection by generating an indirect protection which is additive to the direct protection. So when a TND is used in a population vaccinated with a live influenza vaccine we may underestimate the overall effect of the vaccine.(239) Another potential issue can occur if the vaccine alters 'viral shedding' which may lead to higher number of false negative tests for influenza amongst the vaccinated than unvaccinated individuals. This incidence will violate the assumption of the TND that the rate of non-influenza acute respiratory infections is equal between vaccine groups, and thus will alter VE effect size. However, the inclusion as controls those with a negative test for influenza, but positive for non-influenza respiratory infection aims to remove this potential bias.

The external validity of the TND study can also be questioned since, by definition, it focuses on individuals with acute respiratory illness who then seek healthcare for their symptoms and are swabbed. Thus, according to Westreich et al it is unclear to what extent the results for this study can be generalised to the general population and to those that do not seek care.(307) This PhD work provided evidence on the VE against

influenza infection however the protective effects of the vaccine against clinical outcomes are also important for asthma patients - asthma attacks leading to healthcare consultations or hospital admissions however were not assessed as part of this thesis. One of the initial objectives of this PhD was to assess the VE against clinical outcomes using a retrospective cohort study. However, these data sources from the SIVE II project were only accessible in the third year of my PhD project thus this delay prohibited the assessment of the clinical outcomes in a timely manner. Nevertheless, I still intend to conduct the cohort study and publish these results.

7.4 Integration and reflection of the findings in comparison to existing research activity

The findings in this PhD thesis can be relevant to various national and international research activities which are related with acute or chronic respiratory diseases and preventive strategies. At national level, HPS is a public organisation established by the Scottish Government for the provision of specialist services at national levels such as on respiratory and vaccine preventable diseases including influenza infection.(308) HPS collects data for surveillance purposes on influenza for the monitoring of the influenza activity, vaccine uptake and VE. However, the annual reports on influenza VE estimates produced by Scotland and the UK are not explicitly for asthma, but rather relate to a combined at-risk group (e.g. chronic heart or respiratory diseases).(265)(266) The results of this TND study can provide valuable evidence on the protection that the current influenza vaccines provide in the asthma population. In addition, the close collaboration of the HPS with academic partners involved in the SIVE II project will help to inform them to monitor and identify any gaps on vaccine protection and uptake in specific populations such as asthma. Specifically, the SIVE II project and my study on the asthma population have been used data by HPS with any valuable findings or gaps being reported back to HPS via Dr Jim McMenamin. The AUKCAR is another organisation where the findings of this PhD work are relevant. Specifically, the SIVE II project on asthma is part of AUKCAR's portfolio of work, which aims to reduce asthma exacerbations and prevent asthma-related deaths.(309) New research work from the AUKCAR presented in the Annual Scientific Meeting in Bristol, UK in 2018 also highlighted the importance of the influenza prevention in asthma through influenza vaccination.(310) New research

work revealed that respiratory infections for people with asthma leads to worse asthma outcomes and increased asthma costs.(311) In addition, another research study reported the link between lower socioeconomic status and poor asthma-related healthcare outcomes in Wales.(312) Influenza infection can therefore be connected with both of these findings as it is a common pathogen that causes asthma attacks (details are included in Chapter 1) with higher influenza-induced asthma attacks have been found in socioeconomically deprived populations. Previous work by Sloan et al found an association between lower socioeconomic status and increased risk of hospitalisations due to influenza.(313)

At an international level, the Influenza – Monitoring Vaccine Effectiveness (I-MOVE) network measures the VE annually in Europe where the University of Edinburgh is part of through previous and current work on VE.(126)(128)(314-316) I-MOVE includes a wide scientific community that works on different aspects related to seasonal and pandemic vaccine protection. Specifically, some of the I-MOVE work includes recommendations for the use of the vaccine, provision of more precise VE estimates, improve vaccine composition and use of vaccine boosters (e.g. adjuvants) or booster doses. The findings from the review in this thesis have been already presented to the I-MOVE network during the 10th Annual Meeting in France in 2017. The primary work from this thesis will also give answers to evidence gaps and conflicting evidence on VE highlighted by influenza vaccine scientists from centres in Europe and North America. Specifically, it was agreed that large studies employing data from multiple seasons are required to enhance our current understanding on the protective capacity of the vaccination given the high variability of influenza seasons each year. The primary work in this thesis therefore used data from multiple seasons and enabled the exploration of differences on VE estimates within and across seasons. The methodological limitation of providing pooled VE estimates over multiple seasons was also discussed, arising due to differences in sample size by season and estimates particularly against the A(H3N2) strain (which is more likely to change annually compared to the A(H1N1) and B strains). Differences in VE estimates against the A(H3N2) were also observed in this PhD work.

More studies are also required (as expressed by a representative from the European Centre for Disease Prevention and Control (ECDC) and other scientists) comparing VE estimates between various vaccine types. This is particular important since new vaccine types are introduced or stop being recommended such as the LAIV. The UK is one of the few European countries that it has adopted the LAIV into the childhood immunisation program while USA previously recommended LAIV but not during the 2016/17 and 2017/18 seasons. However, US is now recommending the LAIV for use in children for the 2018/19 influenza season.(194) Evidence from this PhD showed high protection against the influenza B strain from the live vaccine. This adds to the current evidence of VE for LAIV and is supportive of the continuation of the LAIV in the UK. In addition, findings from this thesis can provide evidence on LAIV VE for counties such as US which stopped and have reactivated the LAIV programme.

Role of prior influenza vaccination

The impact of previous vaccination on current VE estimates is another controversial area with various possible explanations from researchers however without a clear answer. It is widely accepted in the scientific community that the impact of pre-existing immunity acquired through either previous influenza infection or vaccination history needs to be assessed through large, long-term prospective studies using virology and immunological data. However, it has been acknowledged that there is a problem of getting enough sample size particularly to individuals with inconsistent vaccination patterns. This PhD work has found that individuals who were vaccinated in previous years were more likely to be re-vaccinated in the current year. Therefore, it is challenging to find an adequate number of individuals that are vaccinated in the current but not in the previous seasons. Researchers from the US and Canada have reported the phenomenon of decreased protection due to repeated vaccination.(317) Possible explanations included the antigenic distance hypothesis where during mismatched seasons (i.e. between the circulating and vaccine strains), the effect of previous vaccination on the current VE estimates is negative.(318) Another possible explanation is the phenomenon of cross-protective immunity that can be induced after a viral exposure and which has been particularly observed between influenza vaccine strains.(319)(320) Thus, the antibody memory from previous vaccinations may have a negative inference with future protection provided by current vaccination. The

infection-block hypothesis is another potential explanation of this negative effect of previous vaccination history. According to Skowronski et al *"prior seasonal vaccination is conjectured to effectively block (protect against) seasonal influenza infection which might have otherwise provided effective heterologous cross-protection against the pandemic strain"*.(321) Findings from this PhD work also found lower VE estimates among individuals with a vaccination history in the previous season. However, there was an overlap of confidence intervals with those individuals with current vaccination only. Finally, unmeasured confounders and characteristics related to patients with asthma could also explain the lower VE estimates among those with current and previous vaccination histories.

Healthcare setting

The healthcare setting where the swab samples are taken for the influenza illness is another area of research that could affect the VE estimates and needs to be investigated. In Scotland prior the 2009 H1N1 pandemic swab samples for influenza detection were predominantly collected in the general practice, however, after the pandemic the number of swabs from hospitals and other secondary health settings increased considerably. The patients' clinical profile differs between outpatient and inpatient settings. Hospitalised patients are older with multiple medical conditions where the VE estimates could be lower due to immunoscenesence or immunosuppression. However, the influenza vaccination could reduce the severity of influenza symptoms and subsequently the number of severe influenza cases requiring hospital admission. The TND study in this thesis included swab samples from both inpatient and outpatient settings. This enabled the assessment of the vaccine protection in a more complete clinical spectrum related to both patients' characteristics and influenza severity. The results in this thesis showed no difference in VE estimates between settings. A result also found during the same seasons (2010-2016) in Northern Spain in adults over 50 years old.(322) Nevertheless, authors claimed that *"in some instances of low VE, vaccination may still reduce the risk of hospitalisation in older adults with vaccine failure"*.(322) This highlights the importance of annual immunisation even in mismatched seasons where the vaccination may not prevent the virus, but it may still

reduce the severity of the influenza infection and subsequently the number of hospital admissions.

TND validation

Observational, analytic studies are widely used in countries with influenza vaccination programmes as the study design of choice for the assessment of the vaccine protection. There has been a rapid increase in the number of TND case-control studies observed in the last decade. According to Sullivan et al *"The chief advantages of this study (TND) over the case-control or cohort design are speed and economy, since it can be nested in routine surveillance, without elevated concerns about the validity of the estimates produced"*.(239) Therefore, two main areas need to be evaluated since the TND study is becoming a reference standard for estimating VE, rather than other observational study designs. First, epidemiological assumptions related to the TND that could potential introduce bias or confounding need now to be assessed given the prominence of the TND study design and its influence on vaccine policy. I have already mentioned in the section 7.3 of this chapter a number of potential bias or confounding that could be inherited with the TND studies if certain assumptions are not satisfied and their potential impact on the VE estimates. Firstly, most of these assumptions are based on theoretical speculations and still need to be confirmed using real-world data. Secondly, the data used for the VE estimates also need to be validated in other studies to ensure the internal and external validity of a TND study. The database used for the primary studies in this thesis were also not validated in previous studies. In my primary studies, data capturing the definition of asthma severity, influenza related hospital admission and influenza related primary care visit was difficult to be determined due to either underuse of relevant codes or the absence of specific codes reflecting these definitions. A recent study found that for asthma diagnosis most clinicians used a large number of definitions and there were even no codes for asthma diagnosis in some instances, but instead asthma-related symptoms were used.(255) The need for standard definitions related to asthma, influenza-related outcomes and influenza vaccination is thus imperative given the extensive use of databases for the provision of the VE estimates. The use therefore of more standard definitions and codes (if possible) will increase the comparability of the VE estimates

at national and international level giving more consistent answers to gaps on VE estimates and increasing the reproducibility of the TND studies.(323)

Vaccine uptake

Another factor that can influence the assessment of the VE is the global low vaccine uptake levels observed in asthma and other at-risk groups. A study measured the influenza vaccine coverage levels based on the number of vaccine doses distributed in 195 countries across all six WHO regions during a 10 year period (2004-2013).(234) According to the study authors, only the UK and Netherlands achieved the desired coverage rate but that was only for older people (≥65 years old). In addition, while the overall distribution in vaccine doses increased up to 87% from 2004 to 2013 the respective increase, from the latter period 2008 to 2013, was only up to 12%. The second phase of this study revealed that barriers related to vaccination included: risk groups' belief that they are not an at-risk group for influenza, lack or doubt about the evidence of vaccine protection and fear about the safety of the vaccination.(234) Suboptimal coverage rates in asthma were also observed in the primary studies in this thesis despite UK being one of the few countries globally achieving optimal uptake levels in the over 65 years old risk group. Evidence from the systematic review and the TND study in this thesis showed that protection from the vaccine can be achieved in the asthma population. In addition, the review in this thesis also confirmed the safety of the inactivated vaccine and the live vaccine especially in older children with asthma. The literature review in Chapter 1 also showed high rates of circulating influenza in the asthma population especially where there is high viral activity as was shown during the 2009 H1N1 pandemic. Additional post-hoc analyses in this thesis embedded in the TND study also found that the asthma population had a higher likelihood of being infected with influenza compared to the other at-risk groups in the TND study of the SIVE II project. Specifically, in patients with asthma, there was a significant odds ratio of being infected with influenza (OR:1.28) compared with (the reference group) non-asthma patients (see Appendix A19; p 332). Potential explanations of this finding could be a) a majority of the swab samples in my TND study were from hospitals were patients will probably have more severe ILI symptoms thus higher chance of having an influenza positive test. In addition, secondary care attendance is more likely to be

sought from individuals living in the most deprived areas in Scotland (325) who may be particularly vulnerable to infectious diseases due to socio-economic reasons as explained below; b) Healthcare providers may recognise which symptoms are related to influenza and take accordingly swab samples which are more likely to be positive for the virus; c) socioeconomic status may also explain the association between asthma and influenza infection susceptibility. Individuals from socioeconomic deprived areas may be more likely to contract influenza due to reasons other than low vaccine uptake. For example, they may live in more crowded houses, be active smokers, living in households with smoking, have poor health literacy and not comply or follow preventive measures during winter seasons. According to the Scottish Public Health Observatory *"A challenge for immunisation programmes is to find effective, simple and inexpensive population wide strategies to recruit hard to reach individuals and those more likely not to take up vaccination opportunities, for example, those who live in areas of greater deprivation, in groups identified as harder to reach and covered by the Equality Act protected characteristics".*(326) Thus, deprivation is a factor that can affect influenza vaccine uptake in the Scottish population and d) differences in the asthma lung epithelium and immune antiviral responses could make asthma patients more vulnerable to the influenza virus compared to other at-risk groups. As a result, findings from this thesis will add more evidence regarding the importance of influenza vaccination in the asthma population and potentially lead to higher uptake rates.

7.5 Implications

7.5.1 Implications for public health immunisation policy in Scotland and in the UK

The UK provides an annual report on the three main parts of the influenza surveillance system which includes results of the monitoring of influenza burden, influenza vaccine uptake and influenza VE. End of influenza season results on the VE estimates are usually provided for various influenza strains, age groups and more recently for vaccine types. The vaccine uptake rates for individuals at-risk for influenza due to a chronic medical condition are provided as 'at-risk ≤65 years old'. No specific information on the asthma population in relation to both the VE estimates and the vaccine uptake rates can be extracted by these annual reports on influenza. Therefore, the research work in this thesis fills a gap between policy and evidence on the VE in

asthma and it also provides stratified and precise evidence on aspects of the influenza vaccine guidelines in the asthma population such as influenza VE estimates by common influenza strains, age groups, influenza vaccine types, swab location and vaccination history. The findings from this thesis will contribute to more effective policy planning by targeting aspects of the immunisation strategy in asthma that could both improve the protective effects of the vaccines and increase their uptake. The implications of this research on the current vaccination policy in the asthma population include:

a) Evidence from the literature review and the primary study showed the vaccines can prevent laboratory-confirmed influenza in both children and adults with asthma. Therefore, this research work supports the continuation of vaccination recommendation in asthma by Scottish and UK immunisation programmes.
b) Lower vaccine protection was observed for the highly mutated influenza A(H3N2) strain in all asthma age groups especially in seasons with antigenic mismatch between the circulating and vaccine strains. Thus, additional methods to supplement vaccine protection should also be adopted (e.g. antiviral prophylaxis, good hand hygiene, follow of an asthma action plan/self-management to avoid attacks,(327) contact of the physician at the early stage of the influenza illness) in seasons that the vaccine fails to provide full protection.
c) Given the high antigenic mutation of the A(H3N2) strain, influenza surveillance systems should use technologies such as genetic sequencing to detect these small mutations of this strain and recommend the right viral strains for inclusion in the vaccines next year.
d) Higher VE estimates against influenza B was found for all age groups compared to influenza A. In addition, high protection of the LAIV was found against influenza B. This finding therefore justifies the introduction and the continuation of the UK LAIV programme in children.
e) A decrease in the VE estimates was observed in people aged 55 and above. This finding is only indicative of the onset of the immune deterioration in older adults. Therefore, future research work is also needed to confirm this finding.

f) The safety of the TIV in asthma was also found in the literature review of this thesis. No safety concerns were also found for the LAIV vaccine in children with asthma. However, more evidence is required for the pre-school children with asthma.

g) Lower vaccine uptake was observed in young adults and in those with less active asthma (i.e. with a past diagnosis for asthma but no active asthma prescriptions). The current guidelines recommend that influenza vaccines be offered for free to people with asthma. Specifically, the UK recommends vaccination for "*asthma that requires continuous or repeated use of inhaled or systemic steroids or with previous exacerbations requiring hospital admission*."(36) Thus, asthma patients with fewer healthcare encounters and not on regular asthma prescriptions may not be targeted for vaccination by the healthcare providers or have irregular patterns of vaccination. Evidence on the protective effects in this asthma group could justify if people with mild or less severe asthma need to be vaccinated resulting in overall higher vaccine coverage and better protection of the asthma population.

7.5.2 Implications for research

This research has helped to reduce some of the uncertainty regarding the protective effects of the main prophylactic measure against influenza in asthma. In addition, new research questions have emerged from this thesis which resulted from assessing various aspects of the vaccine protection and inability to assess all aspects of the vaccine protection within the time framework of this PhD programme. Therefore, the following suggestions will help to fully explore how well the influenza vaccine works in asthma people in real time settings.

a) Additional TND case-control observational studies including multiple influenza seasons are needed to confirm the findings of this research work. In addition, larger sample sizes should be enrolled in order to give more certain answers on the VE in various age groups and between the live and inactivated vaccines.

b) Future TND studies should aim to minimise the effects of bias and unmeasured confounding given that assumptions of this study design are yet to be tested.

c) The severity of asthma status should be carefully defined and included in future VE studies as it is affecting both the propensity of vaccine receipt and influenza-related outcome.

d) Further observational studies need to assess the protective effect of the vaccine in people with asthma that do not require inhaled or systemic steroids or with no history of hospital admissions due to exacerbations. The vaccine is not recommended in people with asthma not currently on ICS or OCS or without a history of previous hospitalisations related to asthma exacerbations in the UK. However, if in seasons with high influenza activity all asthma patients are affected regardless of the severity of their chronic condition, then the above recommendation needs to be re-assessed. Therefore, research that will show both the VE and the burden of the influenza in different asthma severity strata will be required.

e) Retrospective cohort studies are needed to evaluate the VE also against clinical outcomes that are important for people with asthma such as asthma exacerbations leading to emergency visits or hospitalisation. These studies should also include multiple seasons to account for the variability of the influenza seasons and vaccine strains and be powered to fully explore factors that could affect the VE estimates in asthma. In addition, efforts to minimise measured and unmeasured confounding should be made for the provision of more reliable results.

f) The determination of asthma clinical outcomes due to influenza (e.g. hospital admissions, emergency visits, primary care consultations) and asthma severity using electronic health records should be based on valid definitions and algorithms. Studies with no validation assessment of these asthma-related definitions should at least fully describe the process and the algorithms used for these definitions to allow the validity assessment by other researchers. In addition, the supplementary provision of all clinical codes used in the electronic databases is important for the replication of the research findings, validity assessment of the algorithms used and the determination of the best approach to define asthma-related outcomes or severity status based on the available electronic data.(323)

g) Large TND case-control studies should also follow up patients tested positive for influenza during the influenza season to examine the number and type of influenza complications triggered by the influenza virus.
h) Other outcomes of the vaccine protection in asthma should also be evaluated in future studies such as the indirect protection provided particularly by the children to their family members, changes in quality of life and the cost-effectiveness of the vaccine.
i) Further studies to assess the safety of the LAIV in children of all age groups in comparison to the TIV should also be conducted. Concerns regarding the safety of the LAIV in asthma still exist given that the vaccine is not recommended in children with severe asthma or active asthma symptoms.
j) Large cohort observational studies using appropriate statistical models need to be conducted in order to detect which characteristics among asthma people are more strongly associated with vaccine receipt.
k) Qualitative studies are needed to investigate the knowledge, attitudes and beliefs towards influenza vaccination in people with asthma. This would be particularly important in people aged under 65 who reportedly have lower vaccine uptake rates.

7.5.3 Implications for clinical practice

The findings in this thesis provide evidence to healthcare professionals on the benefits of influenza vaccination in people with asthma. The safety of influenza vaccines was also proved in this research work. Findings in this PhD can be fed into risk communication tools that will help clinicians and their patients together make evidence based decisions. Therefore, vaccination will be achieved through a 'shared decision making process' which leads to patient centred medicine.

a) Observational epidemiological studies in the literature and a case-control epidemiologic study using Scottish health-care data has shown that the inactivated and the live influenza vaccines can prevent influenza infection in a proportion of children and adults with asthma. The prevention of the influenza infection is particularly crucial in the asthma patients compared to healthy individuals because it will potentially trigger an asthma attack that, it in the worst-case scenario, could lead to a severe illness requiring intensive care unit

admission or even to death due to secondary complications such as bacterial pneumonia.

b) Higher protection is observed during the seasons where the influenza circulating strains are similar with the strains included in the vaccines. However, the vaccine should still be administered because the inclusion of multiple viral strains means that some degree of protection can still be provided for the other matched concomitant circulating influenza strains.
c) Overall the inactivated and live influenza vaccines appear safe in all age groups. However, more research is needed for the LAIV in younger children.

More efforts to target young adults with asthma and less severe asthma are needed by clinicians as this category of asthma patients are less likely to get vaccinated and be protected against influenza infection and its complications.

7.6 The future of influenza vaccination

The effectiveness of the current influenza vaccines is moderate and ranges between 40-60% during influenza seasons where the vaccine strains are similar to the viral circulating strains.(328)

Although, this level of protection is still important for vulnerable groups of the population such as for those with asthma, more efforts for higher level of protection should be pursued by scientists. Before I list current and future developments in influenza vaccines at national and global level I will discuss some of the problems related to current suboptimal vaccine protection. Indicative factors related to lower protection include: (329)

a) Mutations of the current circulating influenza strains are observed almost every year, which makes it difficult to predict which strains to be included in the vaccines for the next season. These mutations can also occur after the manufacture of the vaccines leading to low protection due to the antigenic distance between the circulating viral types and those including in the vaccine.(330)
b) The current vaccines trigger immune responses by targeting mainly the virus's surface antigen, HA. However, most mutations occur in the HA antigen. Thus,

the virus can escape any immune recognition by existing immune memory from previous or current season vaccination or previous infections.(331)

c) Current inactivated and live vaccines are manufactured in egg cells. However, the virus can still mutate while it is growing in eggs, leading to a vaccine that cannot prevent any circulating strains from infecting a host's cells.(329) In addition, the use of embryonated eggs for the production of these vaccines is slow and it can be a potential problem in future pandemics where demands for large quantities of vaccines in a timely manner is required. In addition, if the pandemic is related to new viral strains where their predominant host is poultry then an additional shortage in embryonated eggs will arise.(330)
d) The immunologic history from multiple exposures to the virus every year from vaccine strains and wild-type circulating strains can also affect the immune response from the vaccine. Thus, immune memory by previous exposure to the virus may block the immune response to any new exposure to viral strains such as influenza B.(317)

Nonetheless new advances in vaccine production and formulation have been reported for the traditional vaccines in the last decade until the long-term solution is achieved (for the above problems) which is translated into a universal influenza vaccine.(332) According to Lambert et al *"The ideal influenza vaccine would be one that is safe, elicits humoral and cellular responses identical to those triggered by a natural infection, provides long-lasting and cross-strain protection, and can be manufactured rapidly in large amounts under well-controlled conditions."*(333) The universal vaccine is a potential vaccine that might replace all influenza vaccines as it is would provide all the qualities described earlier by Lambert at al.(333) The efficacy of a candidate universal vaccine is currently being tested at a world-first clinical trial in the UK which involves people aged 65 and above eligible for vaccination.(334)

However, it is likely to take 10 or more years to bring universal vaccines in the market and recommend them for all currently at-risk groups as they are still in pre-licensure clinical trial phases. Therefore, some of the short-term solutions to improve the protection of the currently licensed vaccines are already available for use.(335) Specifically, the UK is now going to add new vaccine types that could enhance the

efficacy of the current TIVs. In season 2018/19 younger adults aged 18-64 belonging in at-risk group will be offered a quadrivalent inactivated vaccine (QIV) and people over 65 years old will be offered adjuvanted TIV.(336) In Scotland, adults aged 75 years and above were offered adjuvanted TIV due to limited vaccine supply compared to the rest of the UK.(337) The QIV aims to provide better protection by including two influenza B subtypes given that influenza B infection affects more younger age groups, while the adjuvant TIV aims to enhance the immune response in older people that is currently low by the traditional TIVs. New technologies related to the manufacture of the vaccines are also available mostly in the US. Specifically, in 2016 the U.S FDA approved the administration of a quadrivalent cell-based influenza vaccine named 'Flucelvac' in individuals aged four years or older.(338)

A cell-based vaccine technology uses mammalian cells for its manufacture which it is supposed to provide better protection than the egg-based vaccines and their production is rapid since they do not rely on egg supply. Their rapid production is particularly important for pandemics where large demand of the influenza vaccines at short period of times is usually the case.(335) In 2016 the US also approved the administration of a quadrivalent recombinant influenza vaccine named 'Flublok Quadrivalent' in adults aged 18 years or older.(339) The main advantages of this vaccine is that it does not use elements of the influenza virus and does not rely on eggs or mammalian cells during its manufacture process. In addition, the production of these vaccines is faster than the egg or cell-based vaccines.(335) These new developments seem to increase the efficacy of the vaccines based on the pre-licensure clinical trials. Therefore, the assessment of their effectiveness in real world settings through observational studies is now also imperative. The post-market monitoring of the VE and safety will not only establish the continuity of these new vaccines but it should also focus on to identify which at-risk groups will benefit the most of these new vaccine technologies. Therefore, more targeted immunisation recommendations should be aimed by assessing who will be benefited the most by which vaccine types and against which viral strains. To conclude, the efficacy of old and new licensed influenza vaccines is already determined based on their formulation and production process. However, the

effectiveness of the vaccines can still be improved if they are given under conditions that will maximise their protective abilities seen during the pre-licensure phases.

7.7 Conclusions

The systematic review in this thesis revealed for the first time the existence of evidence advocating the protective effects of the influenza vaccination in children and adults with asthma. The primary work in this research also added new evidence about influenza vaccine VE by using a methodologically robust observational study design (TND) and a long follow-up period allowing for various VE assessments. Both the literature and the primary work showed that the vaccines can prevent around 50% of influenza infections. In addition, the systematic review showed that the vaccines prevented 59% to 78% of emergency visits or hospital admissions related to asthma exacerbations. The primary work in this thesis also showed that influenza vaccines provide higher protection during matched seasons, when strains other than the A(H3N2) predominate, when the LAIV is given in place of the TIV in children and in younger age groups less than 55 years old. Evidence about the safety of the vaccination was derived from the literature review which confirmed the safety of the vaccines. Factors related to the vaccine uptake among individuals were also explored based on a 16-year follow-up period. Individuals' characteristics such as females, younger or older age groups for children and adults respectively, co-morbidities, previous vaccination and medical visits history increased the propensity of a vaccine receipt.

Therefore, my results suggest that the research community and policymakers should assess the effectiveness and factors related to the uptake of the vaccine explicitly in the asthma population and not as a risk-group due to a chronic medical condition or chronic respiratory disease. As a result, the extensive investigation of aspects related to VE and vaccine uptake in asthma will improve the vaccination programme's goal to improve the health of the asthma population by preventing or reducing of viral-trigger asthma exacerbations. In addition, the provision of better protection in asthma will also improve the protection of the risk group due to chronic respiratory conditions in general given that most individuals in this category have asthma and COPD and that both conditions can co-exist under the asthma COPD overlap syndrome (ACOS).(340)

The results from the primary work (Chapters 5 and 6) in this thesis also need to be validated and confirmed in other datasets. Future work in other UK or international datasets with similar vaccine recommendations can be used for similar studies in the asthma population. The protective effects of the vaccine against clinical complications related to influenza infections should also be evaluated in future research work. The vaccine protection measured by clinical endpoints is particularly meaningful for both clinicians and asthma patients that could result in higher acceptance and recognition of the value of the vaccination in asthma. In addition, more evidence on the vaccine protection in asthma may increase the current low vaccine coverage. People with asthma need to be annually vaccinated and have optimal vaccination levels in order substantially to reduce the risk of influenza triggered asthma exacerbations and associated morbidity.

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Glossary

Chemokines	They are signaling proteins secreted by cells. Their name is derived from their ability to induce directed chemotaxis in nearby responsive cells.
Cytokines	They are a number of substances, such as interferon, interleukin, and growth factors, which are secreted by certain cells of the immune system and have an effect on other cells.
Immunosenescence	It refers to age-related deterioration of the immune system leading to a decrease in a host's capacity to respond to infections and develop a long-term immune memory, particularly by vaccination.

Appendices

A1. Search Strategies

Medline

1. (chronic disease* or chronic illness* or chronic medical condition*).mp.
2. ((respiratory or pulmonary or lung) and (disease* or illness*)).mp.
3. exp asthma/ or asthma*.mp.
4. ((bronchial* or respiratory or airway or lung*) adj3 (hypersensitiv* or hyperreactiv* or allerg* or insufficiency)).mp.
5. ((bronchial spasm* or bronchospasm* or bronch*) adj3 spasm*).mp.
6. ((bronchoconstrict* or bronch*) adj3 constrict*).mp.
7. or/1-6
8. exp influenza vaccine/
9. ((influenza* or flu) and (vaccin* or immuni* or inocula* or efficacy or effectiveness)).mp.
10. 8 and 9
11. (vaccin* and (monovalent or bivalent or trivalent or quadrivalent or polyvalent or inactivated or live attenuated or recombinant or virosom* or whole or subunit or split)).mp.
12. 10 or 11
13. 7 and 12
14. exp influenza/
15. (influenza* or flu).mp.
16. exp Influenza A virus/ or exp Influenza B virus/
17. 14 and 15 and 16
18. ((laboratory confirmed or laboratory diagnosed) and (influenza* or flu)).mp.
19. (real time polymerase chain reaction or reverse transcript* polymerase chain reaction or RT-PCR or rapid influenza diagnostic test* or fluorescent antibody test* or serologic* test* or viral culture*).mp.
20. (hospitaliz* or hospitalis*).mp.
21. mortality.mp.
22. asthma exacerbation*.mp.
23. (influenza like illness or flu like illness or ILI).mp.
24. (primary care visit* or primary care consultation* or GP visit*).mp.
25. (vaccin* and (adverse event* or adverse effect*)).mp.
26. or/17-25
27. 13 and 26
28. Animals/
29. Humans/
30. 28 not (28 and 29)
31. 27 not 30
32. limit 31 to yr="1970 -Current"

Publication date: 1970 – 22/10/2015

Embase

1. (chronic disease* or chronic illness* or chronic medical condition*).mp.
2. ((respiratory or pulmonary or lung) and (disease* or illness*)).mp.
3. exp asthma/ or asthma*.mp.
4. ((bronchial* or respiratory or airway or lung*) adj3 (hypersensitiv* or hyperreactiv* or allerg* or insufficiency)).mp.
5. ((bronchial spasm* or bronchospasm* or bronch*) adj3 spasm*).mp.
6. ((bronchoconstrict* or bronch*) adj3 constrict*).mp.
7. or/1-6
8. exp influenza vaccine/
9. ((influenza* or flu) and (vaccin* or immuni* or inocula* or efficacy or effectiveness)).mp.
10. 8 and 9
11. (vaccin* and (monovalent or bivalent or trivalent or quadrivalent or polyvalent or inactivated or live attenuated or recombinant or virosom* or whole or subunit or split)).mp.
12. 10 or 11
13. 7 and 12
14. exp influenza/
15. (influenza* or flu).mp.
16. exp influenza virus a/ or exp influenza virus b/
17. 14 and 15 and 16
18. ((laboratory confirmed or laboratory diagnosed) and (influenza* or flu)).mp.
19. (real time polymerase chain reaction or reverse transcript* polymerase chain reaction or RT-PCR or rapid influenza diagnostic test* or fluorescent antibody test* or serologic* test* or viral culture*).mp.
20. (hospitaliz* or hospitalis*).mp.
21. mortality/ or mortality.mp.
22. asthma exacerbation*.mp.
23. (influenza like illness or flu like illness or ILI).mp.
24. (primary care visit* or primary care consultation* or GP visit*).mp.
25. (vaccin* and (adverse event* or adverse effect*)).mp.
26. or/17-25
27. 13 and 26
28. exp animal/
29. human/
30. 28 not (28 and 29)
31. 27 not 30
32. limit 31 to yr="1970 -Current"

Publication date: 1970 – 22/10/2015

CINAHL

#	Query	Limiters/Expanders	Results
S24	S20 not S23	Limiters - Published Date: 19700101-20151031 Search modes - Find all my search terms	3,136
S23	S21 not (S21 and S22)	Search modes - Find all my search terms	56,917
S22	(MH "Human")	Search modes - Find all my search terms	1,341,496
S21	(MH "Animals+")	Search modes - Find all my search terms	63,319
S20	S11 AND S19	Search modes - Find all my search terms	3,587
S19	S15 OR S16 OR S17 OR S18	Search modes - Find all my search terms	2,027,432
S18	"(hospitaliz* or hospitalis* or mortality or asthma exacerbation* or influenza like illness or flu like illness or ILI or primary care visit* or primary care consultation* or vaccin* adverse event* or vaccin* adverse effect*)"	Search modes - SmartText Searching	1,538,335
S17	"(real time polymerase chain reaction or reverse transcript* polymerase chain reaction or rapid influenza diagnostic test* or fluorescent antibody test* or serologic* test* or viral culture*)"	Search modes - SmartText Searching	861,136
S16	"(laboratory confirmed or laboratory diagnosed) and (influenza* or flu)"	Search modes - SmartText Searching	140,859
S15	S12 AND S13 AND S14	Search modes - Find all my search terms	2,647
S14	(MH "Influenza A Virus+") OR (MH "Influenza B Virus")	Search modes - Find all my search terms	3,969
S13	"(influenza* or flu)"	Search modes - SmartText Searching	20,763
S12	(MH "Influenza, Human+")	Search modes - Find all my search terms	5,451

S11	S5 AND S10	Search modes - Find all my search terms	4,185
S10	S8 OR S9	Search modes - Find all my search terms	68,204
S9	"(vaccin*) and (monovalent or bivalent or trivalent or quadrivalent or polyvalent or inactivated or live attenuated or recombinant or virosom* or whole or subunit or split)"	Search modes - SmartText Searching	61,462
S8	S6 AND S7	Search modes - Find all my search terms	7,252
S7	"(influenza* or flu) and (vaccin* or immuni* or inocul* or efficacy or effectiveness)"	Search modes - SmartText Searching	163,623
S6	(MH "Influenza Vaccine")	Search modes - Find all my search terms	7,252
S5	S1 OR S2 OR S3 OR S4	Search modes - Find all my search terms	169,085
S4	"(bronchoconstrict*) or (bronch* adj3 constrict*)"	Search modes - SmartText Searching	285
S3	"(bronchial* spasm* or bronchospasm*)"	Search modes - SmartText Searching	6,192
S2	""(bronchial* or respiratory or airway or lung*) and (hypersensitiv* or hyperreactivit* or allerg* or insufficiency)""	Search modes - SmartText Searching	148,194
S1	"asthma*" OR (MH "Asthma+")	Search modes - Find all my search terms	28,493

Scopus

1 TITLE-ABS-KEY (asthma*)

2 TITLE-ABS-KEY (bronchial* or respiratory or airway or lung*) W/3 (hypersensitive* or hyperreactiv* or allerg* or insufficiency)

3 TITLE-ABS-KEY (bronchial spasm* or bronchospasm*)

4 TITLE-ABS-KEY (bronch* w/3 spasm*)

5 TITLE-ABS-KEY (bronchoconstrict*)

6 TITLE-ABS-KEY (bronch* w/3 constrict*)

7 TITLE-ABS-KEY (or/1-6)

8 TITLE-ABS-KEY (influenza* or flu) and (vaccin* or immuni* or inocul* or efficacy or effectiveness)

9 TITLE-ABS-KEY (vaccine*) and (monovalent or bivalent or trivalent or quadrivalent or polyvalent or inactivated or live attenuated or recombinant or virosom* or whole or subunit or split)

10 TITLE-ABS-KEY (8 or 9)

11 TITLE-ABS-KEY (7 and 10)

12 TITLE-ABS-KEY (influenza * or flu)

13 TITLE-ABS-KEY (laboratory confirmed influenza or laboratory confirmed flu or laboratory diagnosed influenza or laboratory diagnosed flu or polymerase chain reaction or real time polymerase chain reaction)

14 TITLE-ABS-KEY (rapid influenza diagnostic test* or fluorescent antibody test* or serologic* test or viral culture*)

15 TITLE-ABS-KEY (hospitaliz* or hospitalis* or mortality or asthma exacerbation* or influenza like illness or ili or primary care visit* or primary care consultation* or (vaccine* and adverse event*)

16 TITLE-ABS-KEY (or/12-15)

17 TITLE-ABS-KEY (11 and 16)

18 TITLE-ABS-KEY (animal and not (animal and human)

19 TITLE-ABS-KEY (17 not 18)

20 TITLE-ABS-KEY (19) , Pubyear: **1970-2015**, Limit to subjarea: **" Medi", "Immu", "Phar", "Bioc", "Nurs"**

Publication date: 1970 – 23/10/2015

Sciencedirect

Search	Results
pub-date > 1969 and TITLE-ABSTR-KEY(asthma*) *[All Sources(- All Sciences -)]*	34,301
pub-date > 1969 and TITLE-ABSTR-KEY((influenza* or flu) and (vaccin* or immuni* or inocul* or efficacy or effectiveness)) *[All Sources(- All Sciences -)]*	9,857
pub-date > 1969 and TITLE-ABSTR-KEY((vaccin*) and (monovalent or bivalent or trivalent or quadrivalent or polyvalent or inactivated or live attenuated or recombinant or whole or subunit or split)) *[All Sources(- All Sciences -)]*	4,993
(pub-date > 1969 and TITLE-ABSTR-KEY((influenza* or flu) and (vaccin* or immuni* or inocul* or efficacy or effectiveness))) OR (pub-date > 1969 and TITLE-ABSTR-KEY((vaccin*) and (monovalent or bivalent or trivalent or quadrivalent or polyvalent or inactivated or live attenuated or recombinant or whole or subunit or split))) *[All Sources(- All Sciences -)]*	13,896
(pub-date > 1969 and TITLE-ABSTR-KEY(asthma*)) AND ((pub-date > 1969 and TITLE-ABSTR-KEY((influenza* or flu) and (vaccin* or immuni* or inocul* or efficacy or effectiveness))) OR (pub-date > 1969 and TITLE-ABSTR-KEY((vaccin*) and (monovalent or bivalent or trivalent or quadrivalent or polyvalent or inactivated or live attenuated or recombinant or whole or subunit or split)))) *[All Sources(- All Sciences -)]*	168

Publication date: 1970 – 26/10/2015

Web of Science

#14 #11 NOT #12
Refined by: [excluding]: WEB OF SCIENCE CATEGORIES: (THERMODYNAMICS OR MATHEMATICAL COMPUTATIONAL BIOLOGY OR SOCIAL SCIENCES INTERDISCIPLINARY OR REPRODUCTIVE BIOLOGY OR RADIOLOGY NUCLEAR MEDICINE MEDICAL IMAGING OR ZOOLOGY OR PSYCHOLOGY MULTIDISCIPLINARY OR NANOSCIENCE NANOTECHNOLOGY OR NUTRITION DIETETICS OR ENGINEERING MECHANICAL OR DEVELOPMENTAL BIOLOGY OR CONSTRUCTION BUILDING TECHNOLOGY OR INTEGRATIVE COMPLEMENTARY MEDICINE OR CHEMISTRY ORGANIC OR CHEMISTRY MEDICINAL OR AGRICULTURE MULTIDISCIPLINARY OR ENVIRONMENTAL SCIENCES OR WOMEN S STUDIES OR BIOPHYSICS OR TRANSPORTATION OR SOCIAL SCIENCES MATHEMATICAL METHODS OR MEDICAL INFORMATICS OR PSYCHOLOGY CLINICAL OR ECONOMICS OR PHYSICS MULTIDISCIPLINARY OR AGRICULTURE DAIRY ANIMAL SCIENCE OR MYCOLOGY OR METEOROLOGY ATMOSPHERIC SCIENCES OR COMPUTER SCIENCE INTERDISCIPLINARY APPLICATIONS OR MATHEMATICS INTERDISCIPLINARY APPLICATIONS OR PLANT SCIENCES OR MANAGEMENT OR FOOD SCIENCE TECHNOLOGY OR EVOLUTIONARY BIOLOGY OR SUBSTANCE ABUSE OR ENVIRONMENTAL STUDIES OR STATISTICS PROBABILITY OR ENGINEERING ENVIRONMENTAL OR MATHEMATICS APPLIED OR EDUCATION SCIENTIFIC DISCIPLINES OR ECOLOGY OR GENETICS HEREDITY OR COMPUTER SCIENCE SOFTWARE ENGINEERING OR SOCIAL SCIENCES BIOMEDICAL OR SPORT SCIENCES OR ENGINEERING CIVIL OR ENGINEERING CHEMICAL OR OTORHINOLARYNGOLOGY OR ENGINEERING BIOMEDICAL OR ELECTROCHEMISTRY OR EDUCATION EDUCATIONAL RESEARCH OR AGRICULTURAL ECONOMICS POLICY)
DocType=All document types; Language=All languages;

#13 #11 NOT #12
DocType=All document types; Language=All languages;

#12 TS=(animal* NOT (human and animal))
DocType=All document types; Language=All languages;

#11 #10 AND #7
DocType=All document types; Language=All languages;

#10 #9 OR #8
DocType=All document types; Language=All languages;

#9 **TOPIC:** ((laboratory confirmed influenza or hospitali* or mortality or asthma exacerbation* or influenza like illness or primary care visit* or vaccin* adverse event*))
DocType=All document types; Language=All languages;

#8 **TOPIC:** ((influenza* or flu or influenza A virus or influenza B virus))
DocType=All document types; Language=All languages;

#7 #6 AND #3
DocType=All document types; Language=All languages;

#6 #5 OR #4
DocType=All document types; Language=All languages;

#5 **TOPIC:** ((vaccin*) and (monovalent or bivalent or trivalent or quadrivalent or polyvalent or inactivated or live attenuated or recombinant or virosom* or whole or subunit or split))
DocType=All document types; Language=All languages;

#4 **TOPIC:** ((influenza* or flu) and (vaccin* or immuni* or inocul* or efficacy or effectiveness))
DocType=All document types; Language=All languages;

#3 #2 OR #1
DocType=All document types; Language=All languages;

#2 **TOPIC:** ((respiratory or pulmonary or lung) and (disease* or illness*))
DocType=All document types; Language=All languages;

#1 **TOPIC:** (asthma*)
DocType=All document types; Language=All languages;

Publication date: 1970 – 22/10/2015

CENTRAL

ID	Search	Hits
#1	(chronic illness* or chronic disease* or chronic medical condition*):ti,ab,kw in Trials (Word variations have been searched)	34224
#2	(respiratory or pulmonary or lung) and (disease* or illness*):ti,ab,kw in Trials (Word variations have been searched)	25340
#3	asthma*:ti,ab,kw in Trials (Word variations have been searched)	22972
#4	(bronchial* or respiratory or airway or lung*) and (hypersensitiv* or hyperreactivit* or allerg* or insufficiency):ti,ab,kw in Trials (Word variations have been searched)	5636
#5	(bronchial spasm* or bronchospasm* or bronchoconstrict*):ti,ab,kw in Trials (Word variations have been searched)	3153
#6	{or #1-#5}	72409
#7	"influenza vaccine":ti,ab,kw in Trials (Word variations have been searched)	1709
#8	(influenza* or flu) and (vaccin* or immuni* or inocul* or efficacy or effectiveness):ti,ab,kw in Trials (Word variations have been searched)	4392
#9	#7 and #8	1709
#10	(vaccin*) and (monavalent or bivalent or trivalent or quadrivalent or polyvalent or inactivated or live attenuated or recombinant or virosom* or whole or subunit or split):ti,ab,kw in Trials (Word variations have been searched)	4197
#11	#9 or #10	4929
#12	(influenza* or flu or influenza A virus or influenza B virus):ti,ab,kw in Trials (Word variations have been searched)	5711
#13	(laboratory confirmed or laboratory diagnosed) and (influenza* or flu):ti,ab,kw in Trials (Word variations have been searched)	135
#14	(polymerase chain reaction or rapid influenza diagnostic test* or fluorescent antibody test* or serologic* test* or viral culture*)	5449
#15	(hospitaliz* or hospitalis* or mortality or asthma exacerbation* or influenza like illness or flu like illness or ILI or primary care visit* or primary care consultation* or vaccin* adverse event* or vaccin* adverse effect*):ti,ab,kw in Trials (Word variations have been searched)	52562
#16	{or #12-#15}	61654
#17	#6 and #11 and #16 Publication Year from 1970 to 2015, in Trials	408

Publication date: 1970 – 04/11/2015

Cochrane Library

ID	Search	Hits
#1	(chronic illness* or chronic disease* or chronic medical condition*):ti,ab,kw in Cochrane Reviews (Reviews and Protocols) (Word variations have been searched)	970
#2	(respiratory or pulmonary or lung) and (disease* or illness*):ti,ab,kw in Cochrane Reviews (Reviews and Protocols) (Word variations have been searched)	756
#3	asthma*:ti,ab,kw in Cochrane Reviews (Reviews and Protocols) (Word variations have been searched)	259
#4	(bronchial* or respiratory or airway or lung*) and (hypersensitiv* or hyperreactivit* or allerg* or insufficiency):ti,ab,kw in Cochrane Reviews (Reviews and Protocols) (Word variations have been searched)	90
#5	(bronchial spasm* or bronchospasm* or bronchoconstrict*):ti,ab,kw Cochrane Reviews (Reviews and Protocols) (Word variations have been searched)	27
#6	{or #1-#5}	1537
#7	(influenza* or flu) and (vaccin* or immuni* or inocul* or efficacy or effectiveness):ti,ab,kw in Cochrane Reviews (Reviews and Protocols) (Word variations have been searched)	64
#8	(vaccin*) and (monavalent or bivalent or trivalent or quadrivalent or polyvalent or inactivated or live attenuated or recombinant or virosom* or whole or subunit or split):ti,ab,kw in Cochrane Reviews (Reviews and Protocols) (Word variations have been searched)	41
#9	#7 and #8	92
#10	#6 and #9	46

Chinese databases

a) China Knowledge Resource Integrated Database (CNKI)

Chinese search terms:
检索表达式：(TI % '哮喘'OR KY % '哮喘' OR AB % '哮喘') AND (TI % '疫苗' OR KY % '疫苗' OR AB % '疫苗') AND (TI % '流感'+ '感冒' OR KY % '流感'+ '感冒' OR AB % '流感'+ '感冒') 发表时间：从1979-01-01 到2016-01-12

Search terms: (TI % 'xiaochuan' OR KY % 'xiaochuan' AB % 'xiaochuan') AND (TI % 'yimiao' KY % 'yimiao' OR AB % 'yimiao') AND (TI % 'liugan'+ 'ganmao' OR KY % 'liugan'+ 'ganmao' OR AB % 'liugan'+ 'ganmao') ;
Publication date: 01/01/1979 - 12/01/2016
Total: 73

b) Wanfang

Chinese search terms: 检索表达式：(题名或关键词:(哮喘) + 摘要:(哮喘))*(题名或关键词:(疫苗) + 摘要:(疫苗))*(题名或关键词:(流感) + 摘要:(流感) +题名或关键词:(感冒) + 摘要:(感冒)) 时间：1990-2016

Search terms: (TI or KY:(xiaochuan) + AB:(xiaochuan))*(TI or KY:(yimiao) + AB:(yimiao))*(TI or KY:(liugan) + AB:(liugan) + TI or KY:(ganmao) + AB:(ganmao))
Publication date: 1990- 12/01/2016
Total: 53-1=52 (one cannot be accessed)

c) ChongQing VIP

Chinese search terms:
检索表达式：(M=(哮喘)+R=(哮喘))*(M=(疫苗)+R=(疫苗))*(M=(流感+感冒)+R=(流感+感冒)) 时间：1989-2016

Search terms:
(M=(xiaochuan)+R=(xiaochuan))*(M=(yimiao)+R=(yimiao))*(M=(liugan+ganmao)+R=(liugan+ganmao)),
Publication date: 1989- 12/01/2016
Total: 1

World Health Organization Library Information System (WHOLIS)

Search terms:
1. Influenza$ or flu
2. Vaccine$ or immune$
3. 1 AND 2
Publication date: 1970-14/01/2016

Global Health Library (GHL)

Search terms:
1. Influenza, Human (MeSH term)
2. Vaccination (MeSH term)
3. 1 AND 2

Publication date: 1970- 14/01/2016

Trial Registries

a) WHO International Clinical Trials Registry Platform(http://www.who.int/ictrp/en/**)**

Search terms:

1. influenza vaccine*

2. influenza vaccine

3. influenza vaccine AND asthma

Publication date: 01/01/1970 – 21/01/2016

b) ISRCTN registry (http://www.controlled-trials.com/**)**

Search terms:

1. asthma

2. influenza vaccine

3. flu

4. influenza

c) ClinicalTrials.gov (https://clinicaltrials.gov/ **)**

Search terms:

1. influenza vaccine AND asthma
2. flu vaccine AND asthma
3. influenza AND asthma

A2. Characteristics of included studies and summary of risk of bias

Author (year)	Country	Study size (dates)	Age	Clinical setting	Selection Criteria		Intervention		Outcome(s)	Summary risk of bias
					Inclusion	Exclusion				
Randomised Controlled Trials										***Cochrane tool****
Fleming (2006) (128)	Belgium, Finland, Germany, Greece, Israel, Italy, Netherland, Norway, Poland, Portugal, Spain, Switzerlan, UK	2220 (2002-03)	6-17 years	145 study sites in Europe	Clinical diagnosis of asthma with one or more medications	Serious chronic disease, known/suspected immunological disease or current immunosuppresive medication	CAIV-T	TIV	Culture-confirmed influenza illness caused by a subtype similar to that in the vaccine; Culture-confirmed influenza illness caused by any influenza subtype; Respiratory illness outcomes (use of any prescribed medication or antibiotics; unscheduled healthcare provider visits; hospitalisations; absence from school/work); Asthma exacerbation; Acute wheezing illness associated with hospitalisation, unscheduled clinical visit or increased or new asthma medication use; Asthma exacerbation episode within 42 days; PEFR scores pre- and post-vaccination; Daily asthma symptoms; Nighttime awakening scores;	High
Kmiecik (2007) (132)	Poland	286 (2004-05)	18-64 years	Four centres	Atopic asthma and aged	Allergy to any vaccine	TIV	Placebo	Asthma exacerbation; Local & systemic adverse reactions	Unclear

					18-65 years	components, immunocompromised, acute febrile illness in the 72 hours prior inclusion, autoimmune disease, prior immunisation against influenza for the 2004-05 season and influenza vaccine 2 weeks before inclusion or 6 weeks after recruitment.				
Castro (2001) (127)	USA	1952 (2000)	3-64 years	19 lung association	Medical diagnosed stable asthma,	Allergic to egg products or	Trivalent split virus	Placebo	Asthma exacerbation (PEF, asthma medication, visits, hospitalisation);	Unclear

				asthma centres	treatment in the last 12 months and no asthma exacerbations in the last 2 weeks	thimerosal, unable to use the peak flow meter, no telephone, Guillain-Barre syndrome, influenza vaccine the last 6 months, febrile illness within 24h prior recruitment			Asthma-related outcomes(PEFR, asthma symptoms, asthma medication, time loss from work/school due to illness); Injection-associated symptoms	
Pedroza (2009) (137)	Mexico	163 (2001-02)	5-9 years	Outpatient clinics	Mild intermittent and moderate persistent asthma	History of allergy to egg protein or thimerosal	TIV	Placebo	Local & systemic adverse reactions; Pulmonary function(FEV)	Unclear
Miller (2003) (133)	USA	32 (2000)	41-46 years	19 lung association asthma centres	Stable clinical diagnosed asthma and treatment in the	Allergy to egg or thimerosal, Guillain-Barre syndrome, influenza	Inactivated	Placebo	Asthma exacerbation: PEFR, asthma medication, healthcare visits	Unclear

					previous year	vaccine the last 6 months or febrile illness within 24h of recruitment.				
Bueving (2004) (113)	Netherlands	696 (1999-01)	6-18 years	General practice	No details	Other chronic disease, allergy to chicken protein and insufficient understanding of Dutch language.	Inactivated	Placebo	Number, duration and severity of influenza-related asthma exacerbations virologically proven (culture, immunofluorescence, or RT-PCR); Number, duration and severity of influenza-related URT episodes; Number, duration and severity of all URT episodes; Adverse reactions of the vaccination (asthma & URT/LRT symptoms, number, duration and severity of all asthma exacerbations)	Low
Bueving (2004) (126)	Netherlands	696 (1999-01)	6-18 years	General practice	Asthma medication in previous years before recruitment	Other chronic disease, allergy to chicken protein and insufficient understand	Inactivated	Placebo	Local adverse reactions; Systematic adverse effects (Influenza-like illness symptoms, asthma symptoms); Medications for asthma; Airway symptoms; Medical consultations; Absenteeism (school, work)	Unclear

						ing of Dutch language				
Tanaka (1993) (142)	Japan	45 (1989-90)	Mean ± SD(years): 10.1 ± 2.5 (vaccinees), 10.4 ± 2.5 (no vaccine)	Minami Fukuoka national chest hospital	No details	No details	Trivalent recombinant live cold	Placebo/no vaccine	Adverse reactions; Nosocomial outbreak (influenza infection, febrile illness, HI antibody rise)	High
Stenious (1986) (141)	Finland	318 (1981-82)	17-73 years	Outpatient	Moderate to severe asthma, daily medication, normal daily activities, >15 years, ability to make reliable measure	Smokers, history of allergy to egg, immunotherapy or b-adrenergic blocking agents or oral corticosteroids, other co-morbidities	Split-type	Placebo	PEF values; Hospitalisation; Oral steroid medication & daily medication; Asthma symptoms	Unclear

					ments of PEF and keep relevant records, no viral infection s 6 weeks prior the influenza vaccine, stable asthma during the 2 weeks of the study.					
Nicholson (1998) (135)	UK	262 (1995)	19-75 years	9 respiratory centres and 2 asthma clinics	Clinical diagnosis of asthma, stable patients and no need for active revision of medication	Hypersensitivity to eggs, chicken, or influenza proteins, pregnancy, a febrile illness at the beginning of the study and	Trivalent inactivated Split-virus	Placebo	Asthma exacerbations; Pulmonary function (PEF); Cold coinciding with exacerbations; Medication for asthma & respiratory illness; Medical consultations; Hospitalisation for asthma exacerbation	Low

						treatment with an investigational drug during the 30 days before enrolment.				
Sener (1999) (140)	Turkey	24 (1997)	18-69 years	Volunteer patients attending the clinic	No details	Acute respiratory illness, allergy to eggs, pregnancy	Split antigen trivalent	Placebo	Clinical asthma exacerbation or airway hyperreactivity (PEF, PD_{20}, asthma & daily symptoms, asthma medication)	High
Bell (1978) (118)	USA	Phase 1: 79 Phase 2: 93 (1974-75)	6-16 years	Asthma care centre	No details	Allergy to egg	Bivalent	No vaccine	Early adverse pulmonary response (PEFR); Medication for asthma; Hospitalisation (ILI, asthma, ILI & asthma)	High
Reid (1998) (139)	UK	22 (1995)	19-71 years	Not reported	No details	No details	Inactivated subunit	Placebo	Spirometry and airway responsiveness (FEV_1 & PD_{20} methacholine); Asthma or other symptoms; Medication use	Unclear
Redding (2002) (138)	USA	48 (1997)	9-17 years	Three pediatric allergy practices	Diagnosis of stable moderate to severe asthma and with	Daily intranasal corticosteroid, antiviral medications within 2	CAIV-T	Placebo	Pulmonary function (FEV1, PEFR, FVC, FEV1/FVC, FEF); Asthma exacerbation (asthma symptoms, asthma medication, night time awakening score); Post-vaccination symptoms	Unclear

					reversibility (increase in morning FEV1 of >= 12% after use of inhaled albuterol)	weeks of enrolment, hypersensitivity to egg or egg protein, acute febrile illness within 1 week of enrolment, other pulmonary diseases				
Govaert (1993) (130)	Netherlands	25 (1991-92)	≥60 years	General practices	No details	<60 years old, high-risk group, living in an old people's or nursing home.	Split	Placebo	Local & systemic adverse reactions	Unclear
Miyazaki (1993) (134)	Japan	39 (1988-89)	Mean ± SD(years): 11.1 ± 2.7 (vaccinees),	Minami Fukuoka national chest hospital	Inpatients on the asthma ward	Allergy to eggs or chicken feathers	Intranasal cold-adapted recombinant trivalent	No vaccine	Adverse reactions (fever); Febrile illness; Influenza infection	High

			10.0 ± 2.3 (no vaccine)							
Atmar (1990) (125)	USA	17 (1985-88)	36.5 ± 16.8 age range	Healthy volunteers with asthma diagnosis	No details	Acute respiratory illness, allergy to eggs and pregnancy	Live attenuated cold recombinant	Placebo	Pulmonary function (FEV, FVC, FEF) & Histamine bronchoprovocation tests; Respiratory illness (sore throat, rhinorrhoea); Influenza infection; Bronchodilator therapy; Hospitalisation for asthma exacerbation	Unclear
Hahn (1980) (131)	Germany	52 (1979)	N/A	Patients located in Wurzburg	Reversible obstruction of 20% after inhaling Fenoterol, fluctuations in FEV1 of at least 20% when repeatedly measured, definite	No details	Split/ Subunit virus	Placebo	Lung function (FEV1, VC, RAW, TGV, RV, TLC, PF, MEF50, V60); Local and systematic adverse reactions	Unclear

					attacks of breathing difficulty					
Gharagozlou (2007) (129)	Iran	201 (2003)	1-15 years	Specialised clinic of asthma and allergy	No details	Other chronic diseases, Congenital anomalies, Constant use of systemic corticosteroid, no telephone	vaccine	No vaccine	Incidence, frequency and durations of asthma attacks, medication, ED visits, and hospitalisation for asthma attacks; Side effects of the flu vaccine; Absence from kindergarten, due to asthma	Unclear
Ortwein (1987) (136)	Germany	80 (N/A)	N/A	Not reported	No details	No details	Inactivated: whole	Inactivated: split /subunit	Pulmonary function (FEV1); Local and systematic adverse reactions	Unclear
Non-randomised controlled trials										***EPHPP tool*****
Abadoglu (2004) (143)	Turkey	128 (2001-02)	22-84 years	Outpatient clinic	Stable asthma and treatment in the last 12 months	Allergy to egg products, influenza vaccine the last 6 months, febrile illness within 24hr before recruitment	Trivalent inactivated split-virus	No vaccine	Upper respiratory disease; Asthma exacerbations; Hospitalisation (asthma); Pulmonary function (FEV1); Medication for URT infections	Weak

Campbell (1984) (144)	Australia	28 (N/A)	10-70 years	Medical students and outpatients of the respiratory medicine clinic of the royal Brisbane hospital	No details	No details	Subunit trivalent inactivated	Placebo	Asthma exacerbation or asthma symptoms (medication for asthma, pulmonary functions (PEFR), symptoms related to asthma)	Weak
Chiu (2003) (145)	Taiwan	21 (2000)	6-15 years	Outpatient clinics	Stable asthma	Recent ARI or a history of egg allergy	Split antigen trivalent	Placebo	Asthma exacerbation (asthma symptoms, airway responsiveness)	Weak
Kava (1987) (146)	Finland	27 (N/A)	19-53 years	Outpatient	No details	No details	Split virus	Placebo	Symptoms & asthma medication after vaccination; Pulmonary function tests ($R_{aw,}$ ITGV, SG_{aw}); Airway reactivity was measured at each visit by administering aerosols of histamine diphosphate	Weak
Kim (2003) (147)	Korea	16 (N/A)	18-65 years	Outpatient internal clinic	No details	Sensitive reaction to eggs, chicken, or meat, due to pregnancy, new symptoms of	TIV	Placebo	Exacerbation of lung function (PEF); Respiratory symptoms; Asthma medication; Medical consultation	Weak

						upper respiratory tract infection like fever, runny nose, or phlegm at the beginning of the study				
Sugaya (1994) (148)	Japan	137 (1992-93)	2-14 years	Pediatric asthma clinic	No details	No details	Trivalent inactivated subunit antigen	No vaccine	Influenza infection ; Febrile episodes; Asthma attacks; Hospitalisation	Weak
Observational studies										***EPHPP tool****
Christy (2004) (149)	USA	800 (1996-7)	1-19 years	Pediatric teaching practices	No details	No details	Vaccine	No vaccine	Hospitalisations & clinic or emergency visits for asthma or pneumonia diagnosis	Weak
Watanabe (2005) (157)	Japan	115 (2001-03)	15-89 years	Showa University hospital or Ohta-Fukushima general hospital	No details	COPD or influenza vaccine at other hospitals	Vaccine	No vaccine	Asthma exacerbation accompanied by influenza-like illness symptoms	Weak
Smits (2002) (156)	Netherlands	684 (1995-7)	≤12 years old	Primary care database	No details	No details	Trivalent sub-unit	No vaccine	Acute respiratory disease (ILI, bronchitis, bronchiolitis, AE, otitis media)	Moderate

Kramarz (2000) (152)	USA	22231; 38669; 70753 (1993-96)	1-6 years	Computerised outpatient clinic, hospital, ED, and pharmacy files	Asthma diagnosis and Aged 1-6 years	Aged <1 year were excluded because of the difficulty in differentiating between asthma and bronchiolitis in infants <1 year old	Vaccine	No vaccine	Asthma exacerbation (hospitalisation or ED visit)	Weak
Kramarz (2001) (151)	USA	22231; 38669; 70753 (1993-96)	1-6 years	Computerised outpatient clinic, hospital, ED, and pharmacy files	Asthma diagnosis and Aged 1-6 years	Aged <1 year were excluded because of the difficulty in differentiating between asthma and bronchiolitis in infants <1 year old	Vaccine	No vaccine	Asthma exacerbation (hospitalisation or ED visit)	Weak
Jaiwong (2015) (150)	Thailand	93 (2012-13)	1-14 years	Asthma clinic	At least 4 episodes of wheezing in the previous year, positive modified API, low-dose inhaled		Inactivated	No vaccine	Respiratory tract illness; Asthma-related events (wheezing episodes/asthma exacerbation, ED visits, hospitalizations, length of stay for hospitalization & asthma medication)	Weak

					budesonide or oral montelukast 5 mg per day					
Otero (2009) (155)	Spain	338 (2005-06)	6 months –14 years	Primary care centres	No details	Egg allergy or any other component of the vaccine and serious underlying diseases that could alter the effect of vaccination	Vaccine	No vaccine	Influenza; RSV infection Respiratory infection (excluding flu & RSV); Bronchitis; Respiratory crises	Weak
McLean (2014) (153)	USA	1259 (2012-13)	≥6 months	Outpatient clinics	Symptom criteria and duration of illness was <7 days and no antiviral drugs before enrolment	Controls with symptom onset before the 1st date or after the last date of symptom onset among flu cases, Vaccinated within 14 days of illness onset or vaccinated but did not receive all of the ACIP-recommended doses	Vaccine	No vaccine	Vaccine effectiveness against medically-attended ARI and laboratory confirmed influenza (RT-PCR)	Moderate

Ohmit (2014) (154)	USA	566 (2011-12)	≥6 months	Outpatient clinics	ARI on 1 September 2011 and had cough and or fever/feverishness of <7 days	No details	Vaccine	No vaccine	Vaccine effectiveness against medically-attended ARI and laboratory confirmed influenza (RT-PCR)	Moderate

ACIP: Advisory committee on immunization practices; AE: Asthma exacerbation; API: Asthma predictive index; ARI: Acute respiratory infection or illness; CAIV-T: Cold adapted influenza vaccine – trivalent; COPD: Chronic obstructive pulmonary disease; ED: Emergency department; ILI: Influenza-like illness; LRT: Lower respiratory tract; PEF: Peak expiratory flow; RSV: Respiratory syncytial virus; RT-PCR: Real time – polymerase chain reaction; SD: Standard deviation; TIV: Trivalent inactivated vaccine; URT: Upper respiratory tract

*** Risk of bias: low risk of bias, unclear risk of bias, high risk of bias. **Quality assessment: strong, moderate, weak**

A3. Risk of bias assessment of randomised controlled trials

Study ID	Sequence generation	Allocation concealment	Blinding (participants, personnel & outcome assessors)	Incomplete outcome data	Selective outcome reporting	Other sources of bias
Atmar 1990 (125)	Randomisation method not described	No description	Double-blind, but no method description	No missing data reported	Expected outcomes were reported	No report (unclear risk)
Bell 1978 (118)	Randomisation by hospital number	No description	No blinding (cross-over trial)	No missing data reported	Expected outcomes were reported	No details on baseline (high risk)
Castro 2001 (127)	Randomisation using a permuted-block design	Assignment list prepared by the data-coordinating centre	Identical-appearing placebo syringes saline solution, but no details on outcome assessment	96.1% of patients received both injections and completed both 14-day post-injection diaries	Expected outcomes were reported	No report (unclear risk)

Govaert 1993 (130)	Stratified randomisation schedule	No description	Double-blind, but no method description, blind on outcome assessment-questionnaires were analysed by researchers blind to vaccination status	Out of 1838 patients, 1806 had completed data	Expected outcomes were reported	Fewer patients in the older age groups (high risk)
Nicholson 1998 (135)	Computer-generated randomisation code	Sealed envelopes	Assignment codes were concealed until the data had been entered onto the computer and all analytical programs had been tested & identical syringes in appearance and labelling	Out of 287 patients, 255 had complete paired data	Expected outcomes were reported	The primary outcome drove the sample-size calculation and the study was too small to detect significant differences for all the outcomes (high risk)
Pedroza 2009 (137)	Randomisation method not described	No description	Vaccinations were administered by a clinician who was not involved in the assessment of outcomes and the contents of the syringe were shielded from the subject's view, single blind &	No missing data reported	Expected outcomes were reported	No report (unclear risk)

			research nurses and physicians were not aware of the product administered			
Redding 2002 (138)	Randomisation using computer-generated random numbers	No description	Double blind & placebo was administered intranasal as well, no description on outcome assessment	All patients completed the study	Expected outcomes were reported	Small sample size to detect rare adverse events & direct measures of bronchial hyperreactivity were not performed (high risk)
Reid 1998 (139)	Randomisation method not described	Patients were assigned in a double-blind fashion	Double-blind but no description reported	No missing data	Expected outcomes were reported	No report (unclear risk)
Sener 1999 (140)	Randomisation method not described	No description	Single blind, but no report of who was blinded, no blind on outcome assessment	No missing data reported	Expected outcomes were reported	No report (unclear risk)
Stenius 1986 (141)	Randomisation method not described	Double-blind, but no description	Double-blind, used placebo injection, but no description on outcome assessment	318 patients out of 328 completed the first three weeks of the study.	Expected outcomes were reported	No report (unclear risk)

Miyazaki 1993 (134)	Randomisation method not described	No description	No placebo, controls were not vaccinated	No missing data reported	Expected outcomes were reported	No comparable baseline in serology (17/19 vaccines had HI titre >1:64, while only 8/20 non-vaccines had HI titre >1:64 & other baseline details were not reported (high risk)
Tanaka 1993 (142)	Randomisation method not described	No description	Patients were given placebo, but no description	14/45 patients dropped out	Expected outcomes were reported	No baseline characteristics were reported, except age & serology (high risk)
Bueving 2004 (113)	Randomisation using a computer-generated list	Both vaccine & placebo were similar in appearance and manufacturer pack & gave codes to them	All involved subjects were blinded	Missing data for both groups (344/347 vaccinees & 344/349 placebo)	Expected outcomes were reported	No report (unclear risk)
Bueving 2004 (126)	Randomisation using a computer-generated list	Both vaccine & placebo were similar in appearance and	All involved subjects were blinded	No report of missing data	Expected outcomes were reported	Less severe asthmatics, may introduce bias (high risk)

		manufacturer pack & gave codes to them				
Kmiecik 2007 (132)	Randomisation method not described	No description	Double-blind but no description of the external appearance of vaccine & placebo injections	5 out of the 291 randomised subjects dropped out	Expected outcomes were reported	No sufficient baseline characteristics were reported & sponsored by sanofi Pasteur (high risk)
Miller 2003 (133)	Randomisation method not described	No description	Double-blind but no description of the external appearance of vaccine & placebo injections	No missing data reported	Expected outcomes were reported	High type II error due to small sample size and effect sizes (high risk)
Hahn 1980 (131)	Randomisation method not described	No description	No description of the external appearance of vaccine & placebo injections	No missing data reported	Expected outcomes were reported	No report (unclear risk)
Gharazoglou 2007 (129)	Simple, non-restricted randomised selection	No description	No description	No missing data reported	Expected outcomes were reported	No report (unclear risk)

Fleming 2006 (128)	Randomisation method not described	Randomisation was accomplished using an automated interactive voice response system	Open-label trial	Only 9 patients did not complete the study (0.4%)	Expected outcomes were reported	Funding from vaccine companies, manufactured the vaccine in the study (high risk)
Ortwein 1987 (136)	Stratified randomisation	No description	No description	No missing data reported	Expected outcomes were reported	No report (unclear risk)

A4. Overall rating of randomized controlled trials

Study ID	Sequence generation	Allocation concealment	Blinding		Incomplete outcome data	Selective outcome reporting	Overall rating
			Participants & personnel	Outcome assessors			
Atmar 1990 (125)	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Low risk	**Unclear risk**
Bell 1978 (118)	High risk	Unclear risk	High risk	High risk	Unclear risk	Low risk	**High risk**
Castro 2001 (127)	Low risk	Low risk	Low risk	Unclear risk	Low risk	Low risk	**Unclear risk**
Govaert 1993 (130)	Low risk	Unclear risk	Unclear risk	Low risk	Low risk	Low risk	**Unclear risk**
Nicholson 1998 (125)	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	**Low risk**
Pedroza 2009 (137)	Unclear risk	Unclear risk	unclear risk	Low risk	Unclear risk	Low risk	**Unclear risk**
Redding 2002 (138)	Low risk	Unclear risk	Low risk	Unclear risk	Low risk	Low risk	**Unclear risk**
Reid 1998 (139)	Unclear risk	Low risk	Unclear risk	Unclear risk	Low risk	Low risk	**Unclear risk**
Sener 1999 (140)	Unclear risk	Unclear risk	Unclear risk	High risk	Unclear risk	Low risk	**High risk**
Stenius 1986 (141)	Unclear risk	Unclear risk	Low risk	Unclear risk	Low risk	Low risk	**Unclear risk**
Miyazaki 1993 (134)	Unclear risk	Unclear risk	High risk	High risk	Unclear risk	Low risk	**High risk**

Tanaka 1993 (142)	Unclear risk	Unclear risk	Unclear risk	Unclear risk	High risk	Low risk	**High risk**
Bueving 2004 (113)	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	**Low risk**
Bueving 2004 (126)	Low risk	Low risk	Low risk	Low risk	Unclear risk	Low risk	**Unclear risk**
Kmiecik 2007 (132)	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Low risk	Low risk	**Unclear risk**
Miller 2003 (133)	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Low risk	**Unclear risk**
Hahn 1980 (131)	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Low risk	**Unclear risk**
Gharagozlou 2007 (129)	Low risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Low risk	**Unclear risk**
Fleming 2006 (128)	Unclear risk	Low risk	High risk	High risk	Low risk	Low risk	**High risk**
Ortwein 1987 (136)	Low risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Low risk	**Unclear risk**

A5. Non-randomised controlled trials and observational studies

Study ID	Selection Bias (A)		Study Design (B)	Confounders (C)		Blinding (D)		Data Collection Methods (E)		Withdrawals and Dropouts (F)		Intervention Integrity (G)			Analyses (H)			
	Q1	Q2		Q1	Q2	Q1	Q2	Q1	Q2	Q1	Q2	Q1	Q2	Q3	Q1	Q2	Q3	Q4
Christy 2004 (149)	2	5	Cohort analytic	3	4	3	3	3	3	3	4	4	3	6	Individual	Individual	1	3
Jaiwong 2015 (150)	2	5	Cohort analytic	3	4	3	3	3	3	3	4	4	3	6	Individual	Individual	1	3
Smits 2002 (156)	2	1	Cohort analytic	1	2	2	3	3	3	1	1	4	3	6	Individual	Individual	1	3
Watanabe 2005 (157)	2	5	Cohort analytic	3	4	3	3	2	3	2	4	4	3	6	Individual	Individual	2	3
Kramarz 2000 (152)	2	5	Cohort analytic	1	2	3	3	3	3	3	4	4	3	6	Individual	Individual	1	3
Kramarz 2001 (151)	2	5	Cohort analytic	1	2	3	3	3	3	3	4	4	3	6	Individual	Individual	1	3
McLean 2014 (153)	2	5	Case-control	3	4	3	1	1	3	3	5	4	3	6	Individual	Individual	1	3
Ohmit 2014 (154)	2	5	Case-control	3	4	3	1	1	3	3	5	4	3	6	Individual	Individual	1	3
Abadoglou 2004 (143)	2	5	Controlled clinical trial	1	3	3	3	3	3	3	4	4	3	6	Individual	Individual	1	3

Chiu 2003 (145)	2	5	Controlled clinical trial	3	4	3	3	3	3	3	4	4	3	6	Individual	Individual	1	3
Campbell 1984 (144)	2	5	Controlled clinical trial	3	4	3	3	3	3	3	4	4	3	6	Individual	Individual	1	3
Sugaya 1994 (148)	2	5	Controlled clinical trial	3	4	3	3	3	3	3	4	4	3	6	Individual	Individual	1	3
Kava 1987 (146)	2	5	Controlled clinical trial	3	4	3	3	3	3	3	4	4	3	6	Individual	Individual	1	3
Kim 2003 (147)	2	1	Controlled clinical trial	3	4	3	3	3	3	1	1	1	3	6	Individual	Individual	1	3
Otero 2009 (155)	2	5	Cohort Analytic	1	2	3	3	3	3	3	4	4	3	6	Individual	Individual	1	3

A6. Overall rating of non-randomised controlled trials and observational studies

Study ID	Selection Bias (A)	Study Design (B)	Confounders (C)	Blinding (D)	Data Collection Methods (E)	Withdrawals and dropouts (F)	Overall Rating
Christy 2004 (149)	2	2	3	3	3	3	**Weak**
Jaiwong 2015 (150)	2	2	1	2	3	3	**Weak**
Smits 2002 (156)	2	2	2	2	3	1	**Moderate**
Watanabe 2005 (157)	2	2	3	2	3	3	**Weak**
Kramarz 2000 (152)	2	2	2	2	3	3	**Weak**
Kramarz 2001 (151)	2	2	2	2	3	3	**Weak**
McLean 2014 (153)	2	2	3	2	2	2	**Moderate**
Ohmit 2014 (154)	2	2	3	2	2	2	**Moderate**
Abadoglu 2004 (143)	2	1	3	2	3	3	**Weak**
Chiu 2003 (145)	2	1	3	2	3	3	**Weak**
Campbell 1984 (144)	2	1	3	2	3	3	**Weak**
Sugaya 1994 (148)	2	1	3	2	3	3	**Weak**
Kava 1987 (146)	2	1	3	2	3	3	**Weak**
Kim 2003 (147)	2	1	3	2	3	1	**Weak**
Otero 2009 (155)	2	2	2	2	3	3	**Weak**

A7. Grading the quality of evidence

Outcome (No. of studies)	Quality assessment						Summary of findings		
	Study design	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Publication bias**	**Relative effect (95% CI)**	**Quality of evidence (GRADE)**	**Importance of outcome**
Protective effects of influenza vaccination									
Influenza infection (7)	RCTs, non-RCTs, cohort & case-control studies	Serious [1]	Serious [3]	Serious [5]	Some imprecision [7]	Likely [9]	OR 0.55 (95% CI 0.44 to 0.69); RR 0.19 (95% CI 0.06 to 0.67);	Very low	Critical
Asthma exacerbation (7)	RCTs, non-RCTs, cohort studies	Serious [1]	Serious [3]	Serious [5]	Not estimated [8]	Likely [9]	Not estimated [8]	Very low	Critical
Hospitalisation (6)	RCTs, non-RCTs, cohort studies	Serious [1]	Serious [3]	Serious [5]	Not estimated [8]	Likely [9]	Not estimated [8]	Very low	Critical
Primary care consultation (2)	RCT, cohort study	Serious [1]	Serious [3]	Serious [5]	Not estimated [8]	Likely [9]	Not estimated [8]	Very low	Important
Respiratory tract illness or disease (8)	RCTs, Non-RCTs, cohort studies	Serious [1]	Serious [3]	Serious [5]	Some imprecision [7]	Likely [9]	RR 0.28 (95% CI 0.10 to 0.80);	Very low	Critical
Emergency department Visits (3)	RCT, Cohort studies	Serious [1]	Serious [3]	Serious [5]	Not estimated [8]	Likely [9]	Not estimated [8]	Very low	Critical
Medication for asthma (2)	RCT, cohort study	Serious [1]	Serious [3]	Serious [5]	Not estimated [8]	Likely [9]	Not estimated [8]	Very low	Important

Pulmonary function (1)	Non-RCT	Serious [1]	Only one study [4]	Only one study [4]	Only one study [4]	Likely [9]	Not estimated [4]	Very low	Important
School/work absenteeism (1)	RCT	Not serious [2]	Only one study [4]	Only one study [4]	Only one study [4]	Likely [9]	Not estimated [4]	Very low	Important

[1]Studies with unclear or high risk of bias

[2]No studies with unclear or high risk of bias

[3]Various study designs, interventions and outcome definitions

[4]Only one study assessed this outcome, hence consistency, directness and precision of the pooled overall estimated could not be assessed

[5]Comparisons included different type of vaccines, vaccines versus placebo or vaccines versus no vaccines

[6]Both studies compared the safety of inactivated vaccine versus placebo injection

[7]Pooled estimates were precise in only two studies and imprecise estimates were reported in the rest studies

[8]There was heterogeneity (clinical, methodological & statistical) among the studies, hence pooled estimates could not be calculated

[9]Publication bias could not be explored by a statistical test or graphically due to limited number of studies included in meta-analyses

A8. Summary of findings

Outcome (No. of studies)	Study ID (Design)	Intervention		Outcomes		
				Absolute estimates (%)	Relative or VE estimates (95% CI)	Significance
Protective effects of influenza vaccination						
Influenza infection (7)	Fleming (RCT) (128)	LAIV	IV	46/1109 (4.1%) vs. 70/1102 (6.4%)	VE: 35 (4 to 56) (antigenic match)	Significant differenc
	Miyazaki (RCT) (134)	LAIV	No	0/19 (0%) vs. 5/20 (25%)	RR: 0.10 (0.01 to 1.62)	p-value: 0.1
	Tanaka (RCT) (142)	LAIV	No/placebo	2/14 (14.3%) vs. 5/8 (62.5%); 2/14 (14.3%) vs. 8/17 (47.1%)	RR: 0.23 (0.06 to 0.92); RR: 0.30 (0.08 to 1.21)	p-value: 0.04; p-value: 0.1
	Sugaya (Non-RCT) (148)	IV	No	35/85 (42%) vs. 37/52 (71%)	VE: 42.1 % (21 to 57) Age $\geq$ 7 years old: VE: 68% - A(H3N2) VE: 44% - B virus	p-values <0.01
	Otero (Cohort) (155)	Vaccine	No	0/178 (0%) vs. 8/160 (4.4%)	RR: 0.05 (0.003 to 0.91)	p-value: 1.0
	McLean (Case-control) (153)	Vaccine	No	184/387 (48%) vs. 548/872 (63%)	OR: 0.54 (0.42 to 0.68)	p-value <0.0001
	Ohmit (Case-control) (154)	Vaccine	No	30/65 (46%) vs. 290/501 (58%)	OR: 0.62 (0.37 to 1.05)	p-value: 0.08
	Bueving (RCT) (113)	TIV	Placebo	24/347 (7%) vs. 18/349 (5.2%)	RR: 1.31 (0.66 to 2.61)	p-value: 0.44

Asthma exacerbation (7)	Gharagozlou (RCT) (129)	Vaccine	No	39/79 (49.4%) vs. 82/122 (67.2%)	RR: 0.73 (0.57 to 0.95)	p-value: 0.02
	Abadoglou (Non-RCT) (143)	IV	No	15/86 (17.4%) vs. 8/842 (19%)	RR: 0.92 (0.42 to 1.99)	p-value: 0.82
	Sugaya (Non-RCT) (148)	IV	No	One asthmatic attack in the vaccinated group and 18 asthmatic attacks in total during the 1992/93 season. No further details or raw numbers were reported.	Not reported	"No differences in the severity or frequency of asthmatic attack were noted between the two groups"
	Jaiwong (Cohort) (150)	IV	No	Mean (SD): 1.6 (1.6) vs. 6.2 (3.9) (no raw numbers were reported)	Not reported	p-value <0.001
	Kramarz (Cohort) (151)	Vaccine	No	No. of asthma exacerbations: 710; 1,146; 2,564	Incidence rate ratio: 0.78 (0.55-1.10); 0.59 (0.43-0.81); 0.65 (0.52-0.80)	p-value: 0.15; 0 .001; 0.0001
	Watanabe (Cohort) (157)	Vaccine	No	2001/02 season: 2/24 (8.3%) vs. 12/43 (28%) 2002/03 season: 8/57 (14%) vs. 20/58 (35%)	2001/02 season: RR: 0.33 (0.08 to 1.33) 2002/03 season: RR: 0.41 (0.2 to 0.85)	p-value: 0.12; 0.02
Hospitalisation (6)	Bell (RCT) (118)	IV	No	No raw numbers were reported	Not reported	p-value < 0.05
	Fleming (RCT) (128)	LAIV	IV	0 (0.0%) vs. 2 (0.2%) *"The small number of subjects required hospitalization precludes an assessment of efficacy".*	VE: 100.0 (90% CI: -244.7 to 100.0)	No significant difference

	Gharagozlou (RCT) (129)	Vaccine	No	4/79 (5.1%) vs. 8/122 (6.6%)	RR: 0.8 (0.24 to 2.5)	p-value: 0.66
	Abadoglou (Non-RCT) (143)	IV	No	0/86 (0%) vs. 2/42 (4.8%)	RR: 0.1 (0.01 to 2.1)	"No significance"
	Christy (Cohort) (149)	Vaccine	No	0.12 vs. 0.04	OR: 1.9 (0.9 to 3.9)	p-value: 0.10
	Jaiwong (Cohort) (150)	IV	No	Mean (SD): 0.2(0.6) vs. 1.3(1.5) (no raw numbers were reported)	Not reported	p-value <0.001
Primary care consultation (2)	Fleming (RCT) (128)	LAIV	IV	607 (0.7%) vs. 599 (0.7%)	VE: -0.6 (90% CI: -10.8 to 8.6)	No significant difference
	Christy (Cohort) (149)	Vaccine	No	2.14 vs. 0.71	OR: 2.9 (2.0 to 4.1)	p-value < 0.001
Respiratory tract illness or disease (8)	Bueving (RCT) (112)	TIV	Placebo	20/347 (6%) vs. 18/349 (5%)	RR: 0.95 (0.49 to 1.82)	p-value: 0.87
	Miyazaki (RCT) (134)	LAIV	No	0/19 (0%) vs. 6/20 (30%)	RR: 0.08 (0.00 to 1.34)	p-value: 0.08
	Tanaka (RCT) (142)	LAIV	No/placebo	3/14 (21%) vs. 5/8 (63%); 3/14 (21%) vs. 9/17 (53%)	RR: 0.34 (0.11 to 1.07); RR: 0.41 (0.14 to 1.21)	p-value: 0.07; p-value: 0.11
	Abadoglou (Non-RCT) (143)	IV	No	44/86 (51%) vs. 18/42 (43%)	RR: 1.2 (0.8 to 1.8)	"No significance"
	Sugaya (Non-RCT) (148)	IV	No	25/85 (29.4%) vs. 30/52 (57.7%)	VE: 49% (24 to 66)	p-value < 0.01
	Jaiwong (cohort) (150)	IV	No	Mean (SD): 2.2 (2.1) vs. 6.9 (3.9) (no raw numbers were reported)	Not reported	p-value < 0.001

	Otero (cohort) (155)	Vaccine	No	1/178 (0.6%) vs. 4/160 (2.5%); 96/178 (54%) vs. 105/160 (66%); 22/178 (12%) vs. 38/160 (24%)	RR: 0.23 (0.03 to 2); RR: 0.61(0.29 to 0.95); RR: 0.47 (0.26 to 0.84)	p-value: 0.18; p-value < 0.05; p-value < 0.05
	Smits (Cohort) (156)	IV	No	63/293 (22%) vs. 102/391 (26%)	VE: 27% (-7 to 51) (both seasons)	p-value: 0.11
Emergency Department Visits (3)	Gharagozlou (RCT) (129)	IV	No	8/79 (10.1%) vs. 16/122 (13.1%)	RR: 0.77 (0.35 to 1.72)	p-value: 0.52
	Christy (Cohort) (149)	Vaccine	No	0.33 vs. 0.14	OR: 2.0 (1.2 to 3.1)	p-value: 0.01
	Jaiwong (Cohort) (150)	IV	No	Mean (SD): 0.4 (0.9) vs. 2.2 (2.6) (no raw numbers were reported)	Not reported	p-value < 0.001
Medication for asthma (2)	Gharagozlou (RCT) (129)	Vaccine	No	35/79 (44%) vs. 77/122 (63%); 19/79 (24%) vs. 35/122 (29%); (Times per week): 0.87 vs. 1.61	RR: 0.5 (0.36 to 0.66); RR: 0.84 (0.52 to 1.36)	p-value < 0.0001; p-value: 0.47; p-value: 0.027
	Jaiwong (Cohort) (150)	IV	No	Mean (SD): 0.1 (0.3) vs. 1.1 (1.2); 1.6 (1.6) vs. 6.2 (3.9) (no raw numbers were reported)	Not reported	p-value < 0.001
Pulmonary function (1)	Abadoglou (Non-RCT) (143)	IV	No	Mean (SEM): 86.52±2.75 vs. 85.00±4.81	Not reported	No significant difference
School/work absenteeism (1)	Fleming (RCT) (128)	LAIV	IV	1178 (1.4%) vs. 1075 (1.3%) (no. of days)	VE: -9.2 (90% CI: -17.2 to -0.8)	No significant difference

Outcome (No. of studies)	Study ID (Design)	Intervention		Outcomes		
				Absolute estimates (%)	Relative estimates (95% CI)	Significance
Adverse effects of influenza vaccination						
Asthma attacks (8)	Nicholson (RCT) (135)	TIV	Placebo	11/255 (4.3%) (2·2 to 7·6) vs. 3/260 (1.2%) (0·2 to 3·3)	RR: 3.7 (1.1 to 13.2)	p-value: 0.06
	Castro (RCT) (127)	IV	Placebo	Absolute difference: -1.1 (-3.0 to 0.9) (3 days) 1.1 (-1.4 to 3.6) (14 days)	Not reported	No significant difference
	Redding (RCT) (138)	LAIV	Placebo	2/24 (8%) vs. 0/24 (0%)	RR: 5.0 (0.25 to 99.1)	p-value: 0.49
	Miller (RCT) (133)	IV	Placebo	7 vs. 8	Not reported	No significant difference
	Fleming (RCT) (128)	LAIV	IV	31.2% vs. 29.6% (95% CI: -2.2 to 5.4)	Not reported	No significant difference
	Kmiecik (RCT) (132)	IV	Placebo	Difference: 2.8% (95% CI: 1.9% to 4.2%)	Not reported	No significant difference
	Kim (Non-RCT) (147)	IV	Placebo	0% vs. 0%	Not reported	No significant difference
	Kramarz (Cohort) (152)	Vaccine	No	Not reported	Incidence rate ratio: 0.58 (0.36 to 0.95); 0.74 (0.47 to 1.17); 0.98 (0.76 to 1.27)	p-values: 0.03; 0.20; 0.90

Local & systemic adverse reactions (20)	Bueving (RCT) (126)	TIV	Placebo	Local adverse reactions: 28 vs. 9; 69 vs. 23(1999/00) 33 vs. 8; 62 vs. 26 (2000/01) ILI symptoms (signif. only): 14 vs. 6; 37 vs. 27; 24 vs. 6 (1999/00) 3 vs. 8 (2000/01) Asthma symptoms (signif. only): 44 vs. 32	Not reported	p-values: <0.01 (local adverse reactions) 0.02; 0.05; <0.01; 0.02 (ILI symptoms) 0.03(asthma symptoms)
	Fleming (RCT) (128)	LAIV	IV	904 (84.2%) vs. 828 (78.9%) (sys. events); 283 (25.4%) vs. 222 (19.9%) (adverse events)	Not reported	p-values: 0.002 (sys. events); 0.002 (adverse events)
	Gharagozlou (RCT) (129)	Vaccine	No	9/79 (11.4%)	Not reported	Not reported
	Castro (RCT) (127)	IV	Placebo	Mean (SD): 10.4(4.7) vs. 10.4(4.7) (asthma symptom); 25.1% vs. 20.8% (myalgia)	Not reported	p-values: 0.91 (asthma symptom-free days); <0.001 (myalgia)
	Nicholson (RCT) (135)	IV	Placebo	Mean (SD): 19.2(21.6) vs. 16.5(18.9) (all symptoms)	Not reported	All symptoms: p-values: 0.27; 0.01
	Stenius (RCT) (141)	IV	Placebo	No raw numbers were reported	Not reported	Symptom scores for dyspnoea, cough, and production of sputum were

						similar in the two groups.
	Hahn (RCT) (131)	IV	Placebo	Local & systematic symptoms: 16/25 (64%) vs. 9/16 (56%); 12/25 (48%) vs. 9/16 (56%)	RR: 1.14 (0.68 to 1.92); RR: 0.85 (0.47 to 1.5)	The incidence of local and/or systemic symptoms was similar
	Ortwein (RCT) (136)	IV	IV	Local side effect: 12 (9 out of the 12 received subunit vaccine) Systemic reaction: 5 (mainly after split vaccine); 4(after subunit vaccine)	Not reported	p-value: 0.02 (local side effect) 0.04; 0.02 (systemic reaction)
	Redding (RCT) (138)	LAIV	Placebo	7/24 (29%) vs. 8/24 (35%) (asthma symptoms); 22/24 (92%) vs. 21/23 (91%) (local/sys. symptoms)	RR: 0.88 (0.38 to 2.03); RR:1.0 (0.84 to 1.20)	p-value: 0.76 (asthma symptoms); No significant difference
	Sener (RCT) (140)	IV	Placebo	Mean (SD): 4.92 (7.56) vs. 4.66 (7.3)	Not reported	No significant difference
	Miyazaki (RCT) (134)	LAIV	No	4/19 (21%) vs. 3/20 (15%)	RR: 1.4 (0.4 to 5.5)	p-value: 0.62
	Tanaka (RCT) (142)	LAIV	Placeco/No	2/20 (10%) vs. 3/25 (12%) (fever); 16/20 (80%) vs. 13/25(52%) (rhinorrhea)	RR: 0.83 (0.15 to 4.5); RR: 1.54 (1.00 to 2.38)	p-value : No significant difference (fever); 0.05 (rhinorrhea)
	Pedroza (RCT) (137)	IV	Placebo	Local adverse reactions: 55/132 (41.7%) vs. 12/31 (38.7%) (1^{st} dose) 46/132 (34.8%) vs. 9/31 (29%) (2^{nd} dose) Systemic adverse reactions:	RR: 1.1 (0.7 to 1.8); RR: 1.2 (0.7 to 2.2); RR: 0.55 (0.3 to 1.1); RR: 0.73 (0.4 to 1.5)	p-value: (local adverse reactions): 0.76; 0.54 (systemic adverse reactions):

				21/132 (15.9%) vs. 9/31 (29%) (1st dose) 25/132 (18.9%) vs. 8/31 (25.8%) (2nd dose)		0.09; 0.39
	Reid (RCT) (139)	IV	Placebo	9/17 (53%) vs. 0/5 (0%) (transient symptoms) (no asthma symptoms)	Not reported	Not reported
	Govaert (RCT) (130)	IV	Placebo	0/14 (0%) vs. 0/11 (0%) (asthma symptoms)	Not reported	No significant difference
	Kmiecik (RCT) (132)	IV	Placebo	193/286 (67.5%) vs. 55/286 (19.2%) (any local reaction) 162/286 (56.6%) (50.7% to 62.5%) vs. 128/286 (44.8%) (38.9% to 50.7%) (any systemic reaction)	RR: 3.5 (2.7 to 4.5); RR: 1.3 (1.1 tot 1.5)	Significant higher frequency of reactions in the vaccine group than placebo
	Chiu (Non-RCT) (145)	IV	Placebo	Mean: -1.18 vs. -0.50	Not reported	p-values: 0.783; 1.000
	Kim (Non-RCT) (147)	IV	Placebo	Mean (SD): 1.00 (1.93) vs. 0.94 (2.05) (day time symptom) 0.85 (1.58) vs. 1.13 (1.96) (noctural symptom)	Not reported	p-values >0.05
	Kava (Non-RCT) (146)	IV	Placebo	4/16 (25%) vs. 6/11 (54%)	RR: 0.46 (0.2 to 1.3)	p-value: 0.13
	Campbell (Non-RCT) (144)	IV	Placebo	No raw numbers were reported	Not reported	p-value > 0.05
	Bell (RCT)	IV	No	No raw numbers were reported	Not reported	p-value < 0.05

Pulmonary function (16)	(118)					
	Fleming (RCT) (128)	LAIV	IV	No raw numbers were reported	Not reported	No significant differences were observed in mean PEFR findings
	Castro (RCT) (127)	IV	Placebo	Absolute difference: -0.8 (-2.3 to 0.8) (3 days); 0.1 (-1.8 to 2.0) (14 days)	Not reported	No significant difference
	Atmar (RCT) (125)	LAIV	No	1/11 (9%) vs. 2/6 (33%) (FEV_1); 1/11 (9%) vs. 1/6 (17%) (FVC)	RR: 0.3 (0.03 to 2.4); RR: 0.55 (0.04 to 7.3)	p-values: 0.24; 0.64
	Stenius (RCT) (141)	IV	Placebo	No raw numbers were reported	Not reported	No significant difference
	Nicholson (RCT) (135)	IV	Placebo	PEF - change around injection: -2.6 (-6.3 to 1.1) vs. 1.7 (-1.2 to 4.7)	Not reported	p-value: 0.50 vs. 0.73
	Ortwein (RCT) (136)	IV	IV	No raw numbers were reported	Not reported	No significant difference
	Redding (RCT) (138)	LAIV	Placebo	2/24 (8%) vs. 1/24 (4%) (15% or > FEV1 reduction)	RR: 2.0 (0.2 to 21)	p-value: 1.0
	Sener (RCT) (140)	IV	Placebo	Mean (SD): 6.55 (4.97) vs. 6.88 (4.77) (PEF variability)	Not reported	p-value > 0.05
	Pedroza (RCT) (137)	IV	Placebo	Mean: 99.1 vs. 94.2 (FEV1 - visit1); 104.5 vs. 96.2 (FEV1 - visit2)	Not reported	p-values: 0.16; 0.02; No significant differences in FEV1, FEV2, and FEV3
	Reid (RCT) (139)	IV	Placebo	Mean: 2.83 vs. 3.41 (FEV1 - 48h)	Not reported	No significant difference

				2.90 vs. 3.44 (FEV1 - 96h)		
	Hahn (RCT) (131)	IV	Placebo	No raw numbers were reported	Not reported	No significant difference
	Kim (Non-RCT) (147)	IV	Placebo	3 vs. 1 (exacerbation of PFT); Mean (SD): 334 (137) vs. 337 (131) (am PEF); 338 (137) vs. 331 (134) (pm PEF)	Not reported	p-values > 0.05
	Kava (Non-RCT) (146)	IV	Placebo	Median: 0.42 vs. 1.09 (PD_{40})	Not reported	p-value > 0.05
	Chiu (Non-RCT) (145)	IV	Placebo	Mean: 4.99 vs. -3.92 (PEF variability); -98.95 vs. -99.8 (FEV1)	Not reported	p-value: 0.553; 1.000 (PEF variability); 0.286; 0.719 (FEV1)
	Campbell (Non-RCT) (144)	IV	Placebo	Mean difference: -45.3 (morning peak flow) -168.2 (evening peak flow)	Not reported	p-values > 0.05
Medication for asthma (11)	Atmar (RCT) (125)	LAIV	No	No increased use of bronchodilators: 0/11 (0%) vs. 0/6 (0%)	Not reported	No significant difference
	Bueving (RCT) (126)	TIV	Placebo	67/148 (45%) vs. 69/148 (47%) (1999/00) 68/199 (34%) vs. 62/201 (31%) (2000/01)	RR: 0.97 (0.8 to 1.2); RR: 1.1 (0.8 to 1.5)	p-value > 0.05
	Castro (RCT) (129)	IV	Placebo	7% vs. 5.7% (new or increased use of asthma medication)	Not reported	p-value: 0.075
	Nicholson (RCT) (135)	IV	Placebo	Mean(SD):	RR: 1.4 (0.5 to 4.4) (oral steroids)	p-value: 0.85 vs. 0.16 (inh. b2-agonist);

				13.9(11.4) vs. 13.5(10.9) (inh. b2-agonist); 7.4(7.6) vs. 7.2(7.2) (neb. b2-agonist); 7/257 (2.7%) (1.1 to 5.5) vs. 5/258 (1.9%) (0.6 to 4.5) (oral steroids)		0.2 vs. 0.97 (neb. b2-agonist); 0.75 (oral steroids)
	Reid (RCT) (139)	IV	Placebo	No patient reported any increase in medication use: 0/17 (0%) vs. 0/5 (0%)	Not reported	No significant difference
	Bell (RCT) (118)	IV	No	No raw numbers were reported	Not reported	p-value < 0.01
	Sener (RCT) (140)	IV	Placebo	No raw numbers were reported	Not reported	No significant difference
	Stenius (RCT) (141)	IV	Placebo	No raw numbers were reported	Not reported	The need for medication was similar in the two groups.
	Campbell (Non-RCT) (144)	IV	Placebo	Bronchodilator dose: 7 (V > P); 10 (V < P); 9 (V = P)	Not reported	p-value > 0.05
	Kava (Non-RCT) (146)	IV	Placebo	0/16 (0%) vs. 0/11 (0%)	Not reported	No significant difference
	Kim (Non-RCT) (147)	IV	Placebo	Mean (SD): 1.69 (2.41) vs. 1.88 (3.20)	Not reported	p-value > 0.05
Health-care use (5)	Atmar (RCT) (125)	LAIV	Placebo	0/11 (0%) vs. 1/6 (17%)	RR: 0.2 (0.01 to 4.2)	p-value: 0.3
	Bueving (RCT) (126)	TIV	Placebo	0/148 (0%) vs. 1/148 (0.7%) (1999/00)	RR: 0.33 (0.01 to 8.1); RR: 2.0 (0.2 to 22.1)	p-values > 0.05

				2/199 (1.0%) vs. 1/201 (0.5%) (2000/01)		
	Castro (RCT) (127)	IV	Placebo	Absolute difference: -0.5 (-1.3 to 0.3) (3 days) 0.4(-1.0 to 1.8) (14 days)	Not reported	No significant difference
	Nicholson (RCT) (135)	IV	Placebo	1/256 (0.4%) (0 to 2.2) vs. 1/256 (0.4%) (0 to 2.2) (hospital admission); 10/244 (4.1%) (2.0 to 7.4) vs. 7/248 (2.8%) (1.1 to 5.7) (medical consultation)	RR: 1.0 (0.06 to 16); RR: 1.5 (0.6 to 3.8)	p-values: 1.0 (hospital admission) 0.58 (medical consultation)
	Kim (Non-RCT) (147)	IV	Placebo	0% for hospital admission or consultation (no raw numbers were reported)	Not reported	p-value > 0.05
Influenza infection (1)	Atmar (RCT) (125)	LAIV	Placebo	8/11 (73%) vs. 0/6 (0%)	RR: 10 (0.7 to 147)	p-value: 0.1
Respiratory tract illness (1)	Atmar (RCT) (125)	LAIV	Placebo	4/11 (36%) vs. 4/6 (67%)	RR: 0.7 (0.2 to 2.1)	p-value: 0.5
School/work absenteeism (2)	Bueving (RCT) (126)	IV	Placebo	12 vs. 14 (both influenza seasons)	Not reported	p-values > 0.05
	Castro RCT (127)	IV	Placebo	6.7% vs. 6.7%	Not reported	p-value: 1.00

A9. Additional pooled estimates

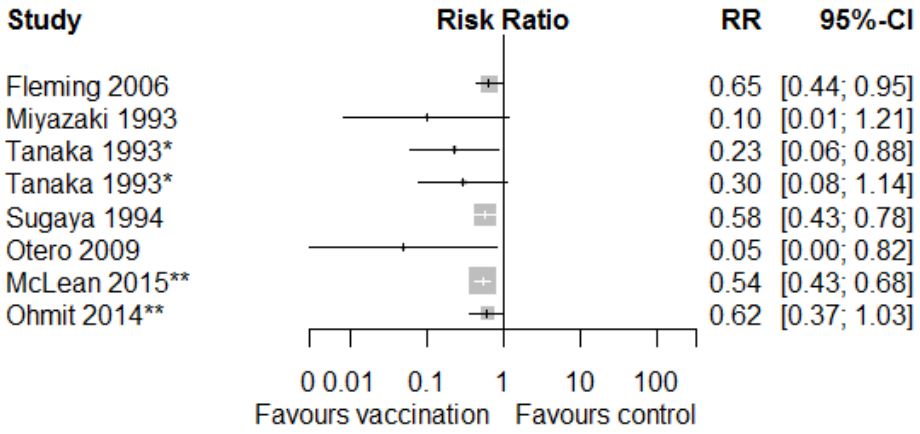

Figure 1: Vaccine effectiveness against influenza infection

*Estimates are from two intervention arms from the same study (Tanaka et al, 1993).

1st comparison is between live vaccine vs. no vaccine and 2nd comparison between live vaccine vs. placebo.

**Effect size in both studies is calculated in ORs and not in RR as implied in the graph.

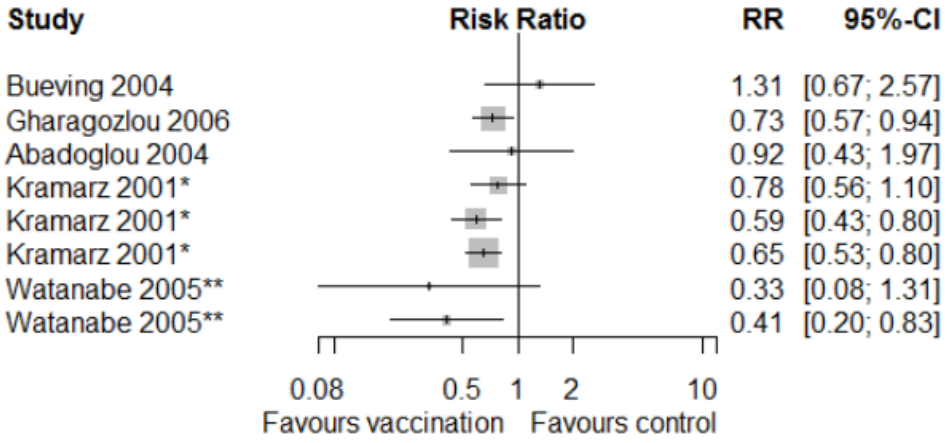

Figure 2: Vaccine effectiveness against asthma exacerbation

*Estimates are from three influenza seasons (1993-96) from the same study (Kramarz et al, 2001).

** Estimates are from two influenza seasons (2001-03) from the same study (Watanabe et al, 2005).

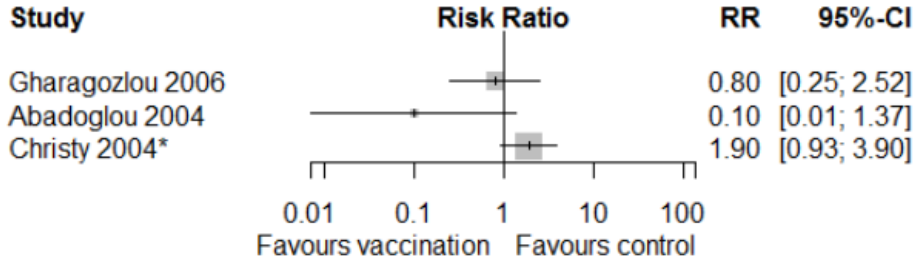

Figure 3: Vaccine effectiveness against hospital admission

*Effect size in study by Christy et al, 2004 is calculated in ORs and not in RR as implied in the graph.

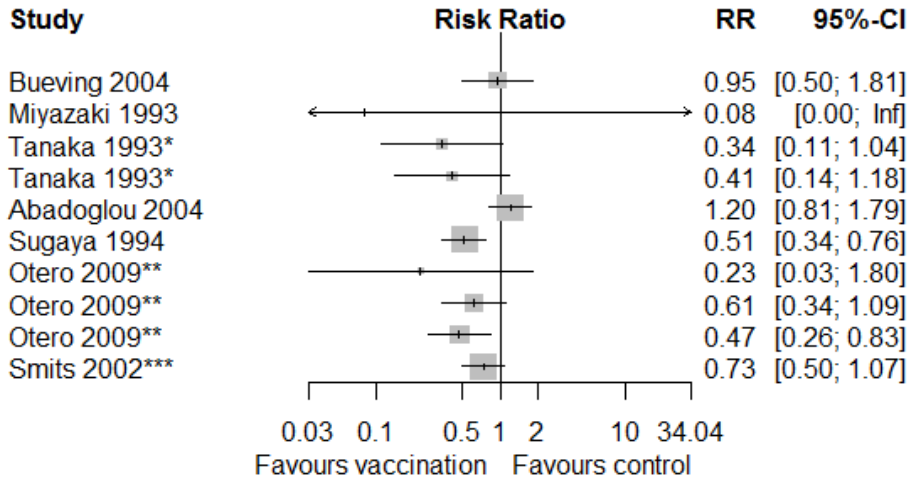

Figure 4: Vaccine effectiveness against respiratory illness

*Estimates are from two intervention arms from the same study (Tanaka et al, 1993).

1st comparison is between live vaccine vs. no vaccine and 2nd comparison between live vaccine vs. placebo.

**Three different respiratory illnesses (RSV infection, other respiratory infections & bronchiolitis) were assessed by the same study (Otero et al, 2009).

***Effect size in study by Smits et al, 2002 is calculated in ORs and not in RR as implied in the graph.

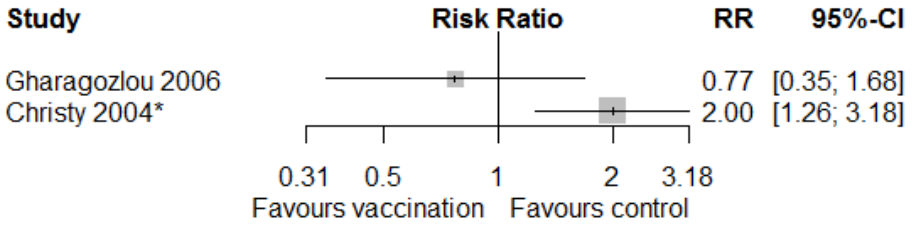

Figure 5: Vaccine effectiveness against emergency visits

***** Effect size in study by Christy et al, 2004 is calculated in ORs and not in RR as implied in the graph.

A10. Further characteristics of included studies

Study	Participants			Interventions		Outcomes
Year & Study design	Enrolment setting	Asthma definition & severity	Exclusion criteria	Source of vaccination data	Definition of vaccine status	Definition
Bell (1978) RCT (118)	Inpatient (asthma care centre)	Reversible obstructive airway disease (test); moderately severe (2/3 on long-term corticosteroids)	Allergy to egg	N/A	N/A	***Primary outcomes:*** -Number of days of hospitalisation per 100 days at risk & the number of patients with influenza-like illness, asthma, influenza-like illness & asthma among hospitalised in each group; ***Secondary outcomes:*** -Early adverse pulmonary response to vaccine measured by PEFR 24 hours following vaccination; -Change in daily count of aerosol treatments with bronchodilator drug per patient in 1st group after vaccination;
Fleming (2006) RCT (128))	Inpatient & outpatient (145 study sites in Europe)	Clinical diagnosis of asthma with 1 or more prescriptions for asthma in the previous 12 months	Serious chronic disease, known/suspected disease of the immune system or current receipt of immunosuppresive therapy (including	N/A	N/A	***Primary outcomes:*** -Incidence of culture-confirmed influenza illness (>=38º C oral temperature, pulmonary congestion, pneumonia or ear infection, 2 or more of the following symptoms; shortness of breath, runny nose or nasal congestion (rhinorrhea), sore throat (pharyngitis), cough, muscle aches,

			high-dose systemic corticosteroids)			chills, headache, irritability, decreased activity, vomiting, increase in wheezing or increased use of medication to treat wheezing) & HA inhibition assay and PCR were used to determine antigenic similarity between flu isolates and the vaccine; -Incidence of culture-confirmed influenza illness caused by any influenza subtype; ***Secondary outcomes:*** -Incidence of hospitalisations or prolongation of existing hospitalisation; --Unscheduled healthcare provider visit or consultation within 28 days of vaccination; -Number of days missed from school or work; -Incidence of asthma exacerbation, defined as acute wheezing illness associated with hospitalisation, any unscheduled clinical visit, or any new prescription (including rescue medication); -PEFR scores during the screening period and for 15 days postvaccination (it was measured 3 times each morning, before any asthma medication was received); -Daily asthma symptoms were assessed in the evening before the subject went

						to sleep and were based on a 4-point scale (0=no symptoms; 1=occasional symptoms; 2=frequent symptoms; and 3=continuous symptoms); -Nighttime awakening scores were assessed each morning using 4-point scale (0=fine; 1=slept well; slight wheeze or cough; 2=awake 2 to 3 times, wheeze or cough; 3=bad night, awake most of the time)
Kmiecik (2007) RCT (132)	Unclear (four centres throughout Poland)	A 12-month history of asthma with perennial symptoms and a positive sprirometry reversibility test, or a positive methacholine or histamine provocation test; atopic asthma	Allergy to any vaccine components, immunocompromised, acute febrile illness in the 72 hours preceding inclusion, autoimmune disease, prior immunisation against influenza for the 2004-2005 season & receive of influenza vaccine 2 weeks before inclusion or 6 weeks after recruitment.	N/A	N/A	***Secondary outcomes:*** -Asthma exacerbations described as either mild or severe according to criteria listed in table 1in the study & daytime and nighttime symptoms of any asthma exacerbations occurring 14 days after each injections were recorded and assigned scores from 0 to 4 according to various criteria listed in table 2 in the study; -Any Local adverse reactions (pain, redness, induration, pruritis, oedema, ecchymosis) & severe local adverse reactions 8 days following injection; Any systemic reactions (asthenia, malaise, headache, sweating, myalgia, arthralgia, shivering, fever) & severe systemic adverse reactions 8 days following injection
Castro (2001) RCT (127)	Unclear (19 Lung Association	Physician-diagnosed asthma;	Allergic to egg products or thimerosal, unable to	N/A	N/A	***Secondary outcomes:*** -Asthma exacerbation within 14 days after an injection defined as the

	Asthma Centres)	mild to moderate asthma	use the peak flowmeter, did not have a telephone, history of the Guillain-Barre syndrome, influenza vaccination the last 6 months, febrile illness within 24h prior			occurrence of one or more of the following: a decrease of at least 30% in the peak expiratory flow rate the second-highest morning peak expiratory flow rate measured during the study; an increase in the daily use of bronchodilator rescue medication above the average use reported in the 2 weeks before randomisation; an increase in the use of systemic corticosteroids for asthma or the addition of systemic corticosteroids to the treatment regime, or the unscheduled use of health care for the treatment of asthma , including a visit to the emergency department, hospitalisation, or a visit or a telephone call to a health care provider; -A decrease of at least 20% in the peak expiratory flow rate from the personal-best rate during the 14 days after each injection; -Symptoms thought to be associated with the vaccine or placebo injection (rhinitis, sore throat, cough, headache, body aches, fever, chills, and fatigue); the number of days without symptoms of asthma; -The amount of time lost from work or school because of illness; -Increase in the dose of a current medication used for long-term control

						of asthma or the addition of such a medication to the treatment regime.
Pedroza (2009) RCT (137)	Outpatient (Outpatient clinics)	Asthma diagnosis based on: daily asthma symptoms, lung function tests, medication for symptoms; mild intermittent and moderate persistent asthma	History of allergy to egg protein or thimerosal	N/A	N/A	***Secondary outcomes:*** -Local adverse events at the inoculation site (pain, erythema, induration) and systemic adverse events (malaise, fever, headache); -Pulmonary function tests FEV_1, FEV_2, FEV_3 at 1, 2, and 3 seconds respectively before and after the flu vaccine
Miller (2003) RCT (133)	Unclear (19 Lung Association Asthma Centres)	Physician-diagnosed asthma and asthma medication within the preceding year; mild to moderate asthma	Allergy to egg or thimerosal, a history of Guillain-Barre syndrome, influenza vaccine the last 6 months or febrile illness within 24h of recruitment.	N/A	N/A	***Secondary outcomes:*** -Asthma exacerbation within 14 days after an injection defined as the occurrence of one or more of the following: a decrease of at least 30% in the peak expiratory flow rate the second-highest morning peak expiratory flow rate measured during the study; an increase in the daily use of bronchodilator rescue medication above the average use reported in the 2 weeks before randomisation; an increase in the use of systemic corticosteroids for asthma or the addition of systemic corticosteroids to the treatment regime, or the unscheduled use of health care for the treatment of asthma , including a visit to the emergency department, hospitalisation, or a visit or a telephone call to a health care provider;

Bueving (2004) RCT (113)	Outpatient (General practice)	Prescription therapy for asthma or more than 52 doses of relief medication during the previous 12 months	Other chronic disease, allergy to chicken protein and insufficient understanding of Dutch language.	N/A	N/A	***Primary outcomes:*** -The number of influenza-related asthma exacerbations virologically proven (culture, immunofluorescence, or RT-PCR) ***Secondary outcomes:*** -The number, duration, and the severity of influenza-related URT episodes & all URT episodes;
Bueving (2004) RCT (126)	Outpatient (General practice)	Prescription therapy for asthma or more than 52 doses of relief medication during the previous 12 months	Other chronic diseases, allergy to chicken protein and insufficient understanding of the Dutch language.	N/A	N/A	***Secondary outcomes:*** -Local adverse reactions 1-7 days after vaccination (erythema at vaccination site, stiff or painful arm); -Influenza-like symptoms 1-7 days after vaccination (sickness/vomiting/diarrhoea, tiredness/sweating, sneezing, runny or stuffed-up nose, burning or watery eyes, sore throat, hoarseness, fever or shivers, headache, myalgia); -Asthma symptoms 1-7 days after vaccination (cough at day/night, wheezing at day/night, dyspnoea); -Medication for airway symptoms or use in total 1-7 days after vaccination; -Consultation of doctor 1-7 days after vaccination; -School /work absenteeism 1-7 days after vaccination;
Tanaka (1993) RCT (142)	Inpatient (Minami-Fukuoka	Institutionalised children with bronchial asthma	Not specified	N/A	N/A	***Primary outcomes:*** -Influenza infection defined by a 4-fold increase or greater HI antibody rise

	National Chest Hospital –on asthma ward)					and/or virus isolation; Virus isolation was performed in Madin-Darby canine kidney cell cultures using pharyngeal swabs collected days 3 and 6 after vaccination from both vaccines and placebo controls; ***Secondary outcomes:*** -Febrile illness defined by a 4-fold increase or greater HI antibody rise and/or virus isolation; -Adverse reactions related to flu vaccine were observed daily for 1 week after inoculation (temperature, asthma attacks and the presence or absence of any subjective symptoms such as rhinorrhea, sore throat, cough, headache, arthralgia or general fatigue were recorded by doctors).
Stenius (1986) RCT (141)	Outpatient	Moderate to severe asthma with daily need of medication and ability to maintain normal activity, all patients fulfilled the criteria for bronchial asthma set by the American College of Chest Physicians and the	Smokers, history of allergy to egg, on current immunotherapy or b-adrenergic blocking agents or oral corticosteroids, other co-morbidities (diabetes, bronchiectasis, chronic bronchitis, emphysema, cancer or chronic collagen diseases).	N/A	N/A	***Secondary outcomes:*** -Adverse reactions (breathlessness, cough, sputum production were assessed daily on a scale of 0 to 3 & fever >37.5ºC, sore throat, symptoms of rhinitis); -PEF values measurements 3 times a day for the first 2 weeks after vaccination and then 2 times a day until the end of April 1982; -Daily medication

		American Thoracic Society.				
Nicholson (1998) RCT (135)	Outpatient (Nine respiratory centres and two asthma clinics in the UK)	Diagnosis by a clinical specialist of recurrent episodes of airway obstruction that resolved on treatment, either during to admission to hospital or during follow-up; stable asthma (requiring no active revision of medication)	Hypersensitivity to eggs, chicken, or influenza proteins, pregnancy, a febrile illness at the beginning of the study and treatment with an investigational drug during the 30 days before enrolment.	N/A	N/A	***Secondary outcomes:*** -Asthma exacerbation occurring 72h of injection, defined by a decline in early-morning PEF of more than 20% compared with the lowest of the best three early-morning PEF values during the 3 days before the injection -PEF mean changes around vaccine and placebo; -Inhaled β2-agonist use 72h before and after injection; -Antibiotic and oral steroid therapy 7 days after each injections; -Unscheduled medical consultations; -Hospital admissions for an exacerbation within 7 days of each injection
Sener (1999) RCT (140)	Outpatient (Volunteer patients attending the clinic)	Patients with mild stable asthma	Acute respiratory illness, allergy to eggs, pregnancy	N/A	N/A	***Secondary outcomes:*** Asthma symptoms (night cough, daytime cough, chest tightness, wheeze, shortness of breath, and sleep disturbance as a result of chest symptoms were recorded to the following scores: 0=absent to 3 =severe); -Daily symptoms (0=did not occue, 1=occurred, but not often or severely enough to cause inconvenience, 2 =interfered slightly with daily

						activities, 3=interfered a lot with daily activities); -Peak expiratory flow (PEF) recording every morning and every evening, complete daily symptom score chart, and record the use of bronchodilator drugs for 1 week; -The lung function measurements and methacoline challenge tests (PD_{20}) were repeated 2 weeks after vaccine and placebo at the same day.
Reid (1998) RCT (139)	Not specified	Patients with stable asthma (all had FEV_1 >60% predicted and >15% reversibility, all tool inhaled β_2 agonists and 20 were on inhaled corticosteroids)	Not specified	N/A	N/A	***Secondary outcomes:*** Spirometry and airway responsiveness (FEV_1 & PD_{20} methacholine) were measured twice a 2 week interval before vaccination and at 48 and 96 hours postvaccination; Asthma or other symptoms; Medication use;
Redding (2002) RCT (138)	Outpatient (3 Pediatric allergy practices)	Asthma defined: FEV_1 <80% after withholding albuterol for 8h, reversibility (>12% increase in morning FEV_1); Stable diagnosed moderate to severe asthma	Daily intranasal corticosteroids, received antiviral medications within 2 weeks of enrolment, history of hypersensitivity to egg or egg protein, acute febrile illness within 1week of enrollment, other pulmonary diseases in addition to asthma.	N/A	N/A	***Secondary outcomes:*** -The difference in values FEV_1(% predicted), FVC(% predicted), FEV1/FVC, $FEF_{25-75\% VC}$ (% predicted) in baseline and 2 and 5 days postvaccination Number of subjects with PEFR $\geq$30% or $\geq$2 SD below baseline & PEFR $\geq$15% or $\geq$2 SD below baseline; Number of days per subject with PEFR $\geq$15% or $\geq$2 SD below baseline; -Asthma exacerbation was defined as asthma symptoms uncontrolled by >6

						puffs of albuterol metered dose inhaler (MDI) during 8h or the need for oral steroids or nebulized bronchodilator treatments; -Daily clinical asthma symptom scores were ranked as 0 to 3 (0=no symptoms; 1=occasional asthma symptoms; 2=frequent asthma symptoms; 3=continuous asthma symptoms); -Nighttime awakening scores were ranked 0 to 3 (0=normal sleep; 1=slept well but with slight wheeze or cough; 2=awoke 2 or 3 times with wheeze or cough; 3=awake most of the time with wheeze) -Post-vaccination symptoms 10 days after vaccination were fever (>100.0^{o}F, oral), cough, sore throat, runny nose, headache, chills, muscle aches and fatigue. Serious adverse events were reported for the 35-day study duration.
Govaert (1993) RCT (130)	Outpatient (General practices)	Not specified	<60 years old, High-risk group, living in an old people's or nursing home.	N/A	N/A	***Secondary outcomes:*** -Local and systemic reactions 48 hours after vaccination were asked through the completion of a questionnaire by patients 4 weeks after injection
Miyazaki (1993) RCT (134)	Inpatient (Minami Fukuoka chest hospital-inpatient ward)	Institutionalized asthmatic children	Allergy to eggs or chicken feathers	N/A	N/A	***Primary outcomes:*** -Influenza infection detected through virus isolation performed in MDCK cell cultures using pharyngeal swabs collected from vaccines who developed body temperature >37.5^{o}C within 1

						week of inoculation and pharyngeal swabs were also collected until March 1989 from all the participants who developed influenza-like illness; (no secondary transmission to non-vaccinees within the same wards was documented, and this was serologically confirmed) ***Secondary outcomes:*** -Adverse reactions such as fever, upper respiratory tract symptoms, and bronchial asthma attack were recorded by doctors daily for 1 week;
Atmar (1990) RCT (125)	Outpatient (Healthy volunteers with asthma diagnosis)	Stable asthma (all had history of intermittent wheezing, 15 participants were on continuous or intermittent bronchodilators)	Acute respiratory illness, allergy to eggs or pregnancy	N/A	N/A	***Secondary outcomes:*** -Influenza infection was defined as virus isolation and/or $\geq$ 4-fold rise in serum NtAb titre between pre and postinoculation serum samples; -Pulmonary function tests, including FEV_1, FVC, FEV_1/FVC and FEF performed 0, 3-4 and 7 postinoculation. A $\geq$ 13% decrease in FEV1 or $\geq$11% decrease in FVC compared to baseline was considered significant in asthmatic volunteers. All tests were performed at the same time on each test day, usually in the morning; Histamine bronchoprovication tests were performed on healthy volunteers; -Clinical evaluation was performed on 0, 2, 3-4 and 7 days after inoculation. A temperature of $\geq$37.8^{o}C was considered

						fever. Signs and symptoms were categorised as mild, moderate or severe by doctors. -Illness was defined as signs and symptoms of an upper and/or lower respiratory illness occurring the first 7 days postinoculation, plus volunteer's perception that he/she had a respiratory illness during the week following postinoculation;
Hahn (1980) RCT (131)	Outpatient (patients located in Wurzburg, Germany)	Reversible airways obstruction, 9 used systemic steroids	Not specified	N/A	N/A	***Secondary outcomes:*** -Local and systematic adverse reactions were measured; -Lung function was determined in the clinical laboratory every 14 days and patients themselves took measurements every day & measurements in the laboratory included: FEV1, VC, RAW, TGV, RV, TLC, PF, MEF50, V60.
Gharagozlou (2007) RCT (129)	Outpatient (patients with asthma referred to the Specialised Clinic of Asthma and Allergy in Tehran)	Asthma diagnosis confirmed by medical examination, difficulty in breathing, coughing and reversible obstruction of respiratory airways (spirometry); about half had intermittent	Comorbidity with other chronic diseases, Congenital anomalies, Constant use of systemic corticosteroids, Lack of telephone	N/A	N/A	***Primary outcomes:*** -Frequency, incidence and duration of asthma attacks; An acute asthma attack was defined as having one or more of the following at one time: increase in β2 agonist use, hospitalisation, use of corticosteroid, visiting medical doctor and emergency room because of asthma; ***Secondary outcomes:*** -Side effects of the flu vaccine (body pain, cough, rhinorrhea, fever, sore throat and malaise, over two weeks following the vaccination);

		asthma, about 30% mild persistent, about 13& moderate persistent and 6% had severe persistent				
Ortwein (1987) RCT (136)	Unclear	Reversible airways obstruction stratified by % in FEV_1	Not specified	N/A	N/A	***Secondary outcomes:*** -Local and systematic adverse reactions; -Pulmonary function (FEV1)
Abadoglou (2004) Non-RCT (143)	Outpatient (patients admitted the outpatient clinic and age/sex matching subjects with asthma was the control group)	Patients took treatment for asthma within the preceding 12 months and had stable asthma ;less than 10% had severe persistent asthma	Allergy to egg products, influenza vaccination the last 6 months, febrile illness within 24hr before recruitment.	N/A	N/A	***Primary outcomes:*** -Exacerbation of asthma was defined as the occurrence of one or more of the following: a decrease of at least 30% in 1-sec forced expired volume (FEV1) from the value measured at the begging of the study, an increase in the daily use of bronchodilator rescue medications (4 or more puffs of a bronchodilator from a metered-dose inhaler or 2 or more uses of nebulized salbutamol for the relief of symptoms), an increase in the use of systemic corticosteroids for asthma or the addition of systemic corticosteroids to the regimen, or the unscheduled visit to the emergency department for the treatment of asthma; ***Secondary outcomes:*** -Frequency of upper respiratory tract infections based on symptoms such as

						fever >37.5ºC, sore throat, cough, body aches, chills and fatigue with no documented underlying disease. The presence and durations of these symptoms was recorded 4 months after the vaccination; -Hospitalisation due to worsening in asthma; - FEV1 measurements before and at the end of the winter.
Campbell (1984) Non-RCT (144)	Outpatient (1/3 were medical students 2/3 were outpatients attending the Respiratory Medicine Clinic of the Royal Brisbane Hospital)	The medical students had mild to moderately severe asthma, and outpatients had had moderate to severe asthma	Not specified	N/A	N/A	***Secondary outcomes:*** -Asthma exacerbation or asthma symptoms was measured through subjective data such as patients' perception of their symptoms related to asthma including: night wheeze, cough, day wheeze, sputum, activity level during the day and the total number of puffs of bronchodilator dose used in last 24 hours and objective data such as measurements of peak expiratory flow rate (PEFR) twice daily;
Chiu (2003) Non-RCT (145)	Outpatient (children with stable asthma attending clinics)	The diagnosis and severity of asthma was made according to the Guidelines of Global Initiative on Asthma (GINA)	Recent acute respiratory illness or a history of egg allergy	N/A	N/A	***Secondary outcomes:*** -Asthma symptoms, including night cough, daytime cough, chest tightness, wheeze, shortness of breath, and sleep disturbance were recorded according to the following scores: 0=absent to 3=severe; -Daily symptoms were 0=did not occur, 1=occurred, but not often or severely enough to cause

						inconvenience, 2=interfered slightly with daily activities, and 3=interfered a lot with daily activities; -Daily PEF variability was calculated according to a formula (it is provided in the paper); -FEV_1 and methacholine challenge test (PC_{20}) were performed on day 1 pre-vaccination and repeated on day 14 post-vaccination in both groups; Patients in both groups were requested to record their PEF every morning and every evening and to complete a daily symptom score chart for 2 weeks;
Kava (1987) Non-RCT (146)	Outpatient	Clinical history and bronchial hyperreactivity to inhaled diphosphate test; 20 had mild and 7 moderately severe asthma	Not specified	N/A	N/A	***Secondary outcomes:*** -Symptoms records such rhinorrhoea, cough, sore throat, increased dyspnoea, fever, headache, muscle or joint pain, and general discomfort at 2, 3 and 21 days after vaccination and between visits were recorded as well as consumption of bronchodilator drugs; -Pulmonary function tests such as airway resistance (R_{aw}) and intrathoracic gas volume (ITGV), of which SG_{aw} before and 2, 3 and 21 days after vaccination; Airway reactivity was measured at each visit by administering aerosols of histamine diphosphate with a No. 40 de Vilbiss nebuliser;

Kim (2003) Non-RCT (147)	Outpatient (patients who had visited the outpatient internal clinic)	Bronchial asthma: symptoms (difficulty in breathing, cough), increase by more than 15% of FEV_1 after Ventolin or when PC_{20} was less than 25mg/ml from the methacholine challenge test; Severity of asthma based on GINA, 3 had severe persistent asthma	Sensitive reaction to eggs, chicken, or meat, due to pregnancy, new symptoms of upper respiratory tract infection like fever, runny nose, or phlegm at the beginning og the research	N/A	N/A	***Secondary outcomes:*** -Daytime symptoms were measured as follows: 0=no asthmatic symptom, 1=asthmatic symptom but did not affect the daily activities, 2=daily activities were affected a small amount by asthmatic symptoms, 3=daily activities were affected most of the time by asthmatic symptoms; -Evening symptoms were defined as the number of times the patient woke up during their sleep due to asthmatic symptoms and were recorded by patients; -Patients' daily diary card on their clinic visits and recorded any extra outpatient clinic and ER visits, or increased use of additional medication due to the worsening of the asthmatic symptoms were checked by researchers; -Effect of influenza vaccine on pulmonary function 72h after vaccination in stable asthma was measured comparing the result of amPEF 3 days before the shot. A decrease of more than 20% first and secondly pmPEF in decrease, was considered that the flu shot would be the cause of worsening of the lung function;

						-The number of times that fast-acting bronchodilator Ventolin, Bricanyl was used was recorded by participants.
Sugaya (1994) Non-RCT (148)	Outpatient (pediatric asthma clinic)	Patients with moderate to severe asthma	Not specified	N/A	N/A	***Primary outcomes:*** -Influenza infection was established when a virus was isolated and/or the individual showed a 4-fold or greater increase in HI antibody titer to the epidemic influenza type A or B; Blood sample were drawn from the vaccines 3 to 4 weeks after the 2nd vaccination and HI tests were performed; Virus isolation was performed when the subjects came to the hospital with complaints of fever or respiratory symptoms, such as cough, rhinorrhea, and sore throat, specimes for isolation were collected by throat swabs and placed in balanced salt solution supplemented with 100μg/mL of gentamicin. ***Secondary outcomes:*** -Febrile episodes with antibody rise or virus isolation; The diagnosis of febrile illness was based on fever 38.0°C or higher for 2 days or fever 39.0°C or higher for 1 day during the period Dec. 15, 1992 ro Feb. 28, 1993;
Christy (2004) Cohort	Outpatient (enrolled in two large paediatric	Asthma severity was based on medical records	Not specified	Data were obtained from computerised	Not specified	***Secondary outcomes:*** -Mean annual number of hospitalisations and visits to the clinic

(149)	teaching practices serving children of low to moderate income- data were obtained from computerised administrative databases and verified where possible by chart review)	and categorised as "mild, moderate, or severe", When severity was not specified, asthma medication was used as a proxy of severity		administrative databases and verified where possible by chart review		or emergency department (ED) for the diagnosis of asthma and pneumonia;
Watanabe (2005) Cohort (157)	Outpatient (patients at the Respiratory Division of Showa University Hospital or Ohta-Fukushima general hospital)	Asthma was defined as recurrent episodes of airway obstruction that resolved on treatment; severity of asthma based on daily medication according to GINA	Patients with chronic obstructive pulmonary disease (COPD) or being vaccinated against influenza at other hospitals	Information based on medical records	Not reported	***Primary outcomes:*** -Asthma exacerbation was defined as asthma attacks accompanied by wheezing or a decrease of more than 20% in PEF; The number of asthma exacerbation accompanied by ILI were calculated per person; -Influenza-like illness symptoms associated with influenza infection according to patients' symptoms using a scoring methos. Each symptom, such as sore throat or rhinorrhea, fever, general fatigue, cough, and sudden onset, was assigned 1 point, and a total of 3 or more points were defined as ILI;
Smits (2002) Cohort (156)	Outpatient	Asthma criteria defined in the	Not specified	Data were extracted	Not reported	***Secondary outcomes:***

	(patients registered to a primary care database in 1995)	guidelines of the Dutch College of General Practioners;		anonymously from electronic patient records and classified by a physician		-One or more episodes of acute lower respiratory tract disease defined as physician-diagnosed influenza-like illness, pneumonia, bronchitis, bronchiolitis, asthma exacerbation or acute otitis media during the influenza seasons;
Kramarz (2000) Cohort (152)	Outpatient & inpatient (computerised outpatient clinic, hospital, ED, and pharmacy files)	Asthma case had to meet 1 of the criteria: at least 1 ICD-9 code 493 and at least 1 prescription for any asthma medication, or at least 1 prescription for a β-agonist drug and at least 1 for cromolyn, or >5 prescriptions for any asthma medication; severity based on β-agonists prescriptions and the no. of hospitalisations and ED visits for asthma 6 months before the flu season	Children younger than 1 years old were excluded because of the difficulty in differentiating between asthma and bronchiolitis in infants <1 year old	Data from computerised vaccination databases	Not reported	***Secondary outcomes:*** -Asthma exacerbation was defined as a hospitalisation or ED visit for asthma indentified from the computerized HMO databases (with 2-day and 2-week interval after vaccination).
Kramarz (2001) Cohort (151)	Outpatient & inpatient (computerised outpatient	Asthma case had to meet 1 of the criteria: at least 1 ICD-9 code 493	Children younger than 1 years old were excluded because of the difficulty in	Data from computerised vaccination databases	Not reported	***Primary outcomes:*** -Asthma exacerbation was defined as a hospitalisation or ED visit for asthma indentified from the computerized

	clinic, hospital, ED, and pharmacy files)	and at least 1 prescription for any asthma medication, or at least 1 prescription for a β-agonist drug and at least 1 for cromolyn, or >5 prescriptions for any asthma medication; severity based on β-agonists prescriptions and the no. of hospitalisations and ED visits for asthma 6 months before the flu season	differentiating between asthma and bronchiolitis in infants <1 year old.			HMO databases (no 2-week interval after vaccination).
Jaiwong (2015) Cohort (150)	Outpatient (asthma clinic)	At least 4 episodes of wheezing in the past year, positive modified Asthma Predictive Index (API), low-dose inhaled budesonide or oral montelukast; mild-persistent asthmatic children	Not specified	N/A	Children received 2 doses of vaccine intramuscularly at 1 month interval	***Primary outcomes:*** -Asthma exacerbations defined as wheezing episodes. ***Secondary outcomes:*** -The rate of acute respiratory tract illness; -Emergency room visits; -Hospitalizations, length of stay for hospitalization; -Bronchodilator usage and systemic steroid and both groups were followed up every 3 months at the asthma clinic;
Otero (2009) Cohort (155)	Outpatient	Patients diagnosed with asthma at the	Egg allergy, to any other component of	N/A	Not reported	***Primary and secondary outcomes:***

	(primary care centres)	centres; Severity of asthma based on criteria of the Spanish Guide for Handling and about 50% had occasional episodic asthma and the rest 50% episodic frequent to moderate persistent asthma	the vaccine or those with serious underlying disease that could alter the effect of vaccination (inencia, deficit of immunoglobulin, heart disease, bronchopulmonary dysplasia, cystic fibrosis, pathology neuro system			-Respiratory infections were recorded and episodes of bronchitis that presented the patients during the flu and clinical criteria were used (cough, fever) associated with laboratory confirmation.
McLean (2014) TND (153)	Outpatient (patients with acute illness for $\leq$7 days were enrolled and had swab samples taken at outpatient clinics)	Chronic disease was based on the ICD-9 diagnosis codes from the electronic medical record, they were at high-risk if they had a healthcare encounter leading to an ICD-9 code corresponding to a high-risk condition the year before enrolment	Controls with symptom onset before the 1st date or after the last date of symptom onset among flu cases, Vaccinated within 14 days of illness onset or vaccinated but did not receive all of the ACIP-recommended doses	Medical/registry documented receipt of current-season vaccine	Persons $\geq$ 9 years were considered vaccinated, given documented receipt of at least 1 dose of current-season vaccine $\geq$14 days before illness onset and children aged 6 months to 8 years were considered vaccinated if they received all the recommended	***Primary outcomes:*** -Cases were defined as persons with a medically attended ARI confirmed as influenza by RT-PCR. Controls were persons with a medically attended ARI who tested negative for influenza virus.

					doses by ACIP, based on their documented vaccination history	
Ohmit (2014) TND (154)	Outpatient (patients with acute illness for ≤7 days were enrolled and had swab samples taken at outpatient clinics)	The current health status based on interview and subjects were defined as high risk if they had medical record documentation the past year prior enrolment	Not reported	Vaccination status was based on medical records or immunisation registries	Subjects were considered vaccinated if they had documented evidence of receipt at least 1 dose of vaccine for the current season at least 14 days before illness onset	***Primary outcomes:*** -Cases were defined as persons with a medically attended ARI confirmed as influenza by RT-PCR. Controls were persons with a medically attended ARI who tested negative for influenza virus.

A11. Indicators of the seven domains of the Scottish Index of Multiple Deprivation (SIMD)

Domain	Indicators
Income	Income Support and Income-based Employment Support Allowance claimants (16-59)
	Job Seekers Allowance and Guaranteed Pension Credit Claimants (All ages)
	Universal Credit claimants with no employment marker
	Number of children in JSA, IS or ESA households
	Number of Adults and children dependent on adults in receipt of tax credits
Employment	Unemployment Claimant Count averaged over 12 months
	Working age Incapacity Benefit or Employment Support Allowance recipients
	Working age Severe Disablement Allowance recipients
Health	Standardised Mortality Ratio
	Hospital stays related to alcohol misuse
	Hospital stays related to drug misuse
	Comparative Illness Factor
	Emergency stays in hospital
	Proportion of population being prescribed drugs for anxiety, depression or psychosis
	Proportion of live singleton births of low birth weight
Education, Skills and Training	School pupil attendance
	School pupil performance
	Working age people with no qualifications
	17-21 year olds enrolling into full time higher education
	School leavers aged 16-19 not in education, employment or training
Housing	Persons in households which are over-crowded
	Persons in households without central heating
Geographical access to services	Drive time sub-domain (weight = 2/3)
	Drive time to GP, to retail centre, to petrol station, to primary and secondary schools, to post office
	Public transport sub-domain (weight = 1/3)
	Public transport time to GP, to retail centre, to post office
Crime	Domestic house breaking
	Drug offences
	Common assault
	Crimes of violence
	Vandalism
	Sexual offences

A12. STROBE Checklist

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls
		(*b*) For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how matching of cases and controls was addressed
		(*e*) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed

		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

A13. RECORD Checklist

	Item No.	STROBE items	Location in manuscript where items are reported	RECORD items	Location in manuscript where items are reported
Title and abstract					
	1	(a) Indicate the study's design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found		RECORD 1.1: The type of data used should be specified in the title or abstract. When possible, the name of the databases used should be included. RECORD 1.2: If applicable, the geographic region and timeframe within which the study took place should be reported in the title or abstract. RECORD 1.3: If linkage between databases was conducted for the study, this should be clearly stated in the title or abstract.	p. 106
Introduction					
Background rationale	2	Explain the scientific background and rationale for the investigation being reported			p. 106-108
Objectives	3	State specific objectives, including any prespecified hypotheses			p. 107-108
Methods					

Study Design	4	Present key elements of study design early in the paper			p. 108-110
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection			p. 108-110
Participants	6	*(a) Cohort study* - Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study* - Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study* - Give the eligibility criteria, and the sources and methods of selection of participants *(b) Cohort study* - For matched studies, give matching criteria and		RECORD 6.1: The methods of study population selection (such as codes or algorithms used to identify subjects) should be listed in detail. If this is not possible, an explanation should be provided. RECORD 6.2: Any validation studies of the codes or algorithms used to select the population should be referenced. If validation was conducted for this study and not published elsewhere, detailed methods and results should be provided. RECORD 6.3: If the study involved linkage of databases, consider use of a flow diagram or other graphical display to demonstrate the data linkage process, including the number of individuals with linked data at each stage.	p. 108-114

		number of exposed and unexposed *Case-control study* - For matched studies, give matching criteria and the number of controls per case			
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable.		RECORD 7.1: A complete list of codes and algorithms used to classify exposures, outcomes, confounders, and effect modifiers should be provided. If these cannot be reported, an explanation should be provided.	p. 115-117
Data sources/ measurement	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group			p. 115-117
Bias	9	Describe any efforts to address potential sources of bias			p. 120
Study size	10	Explain how the study size was arrived at			p. 121
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe			p. 115-117

		which groupings were chosen, and why			
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (d) *Cohort study* - If applicable, explain how loss to follow-up was addressed *Case-control study* - If applicable, explain how matching of cases and controls was addressed *Cross-sectional study* - If applicable, describe analytical methods taking account of sampling strategy (e) Describe any sensitivity analyses			p. 117-121
Data access and cleaning methods		..		RECORD 12.1: Authors should describe the extent to which the investigators had access to the database population used to create the study population.	p. 110

				RECORD 12.2: Authors should provide information on the data cleaning methods used in the study.	
Linkage		..		RECORD 12.3: State whether the study included person-level, institutional-level, or other data linkage across two or more databases. The methods of linkage and methods of linkage quality evaluation should be provided.	p. 110
Results					
Participants	13	(a) Report the numbers of individuals at each stage of the study (*e.g.*, numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed) (b) Give reasons for non-participation at each stage. (c) Consider use of a flow diagram		RECORD 13.1: Describe in detail the selection of the persons included in the study (*i.e.*, study population selection) including filtering based on data quality, data availability and linkage. The selection of included persons can be described in the text and/or by means of the study flow diagram.	p. 123
Descriptive data	14	(a) Give characteristics of study participants (*e.g.*, demographic, clinical, social) and information on exposures and potential confounders			p. 123-126

		(b) Indicate the number of participants with missing data for each variable of interest (c) *Cohort study* - summarise follow-up time (*e.g.*, average and total amount)			
Outcome data	15	*Cohort study* - Report numbers of outcome events or summary measures over time *Case-control study* - Report numbers in each exposure category, or summary measures of exposure *Cross-sectional study* - Report numbers of outcome events or summary measures			p. 127-131

Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period			p. 127-131
Other analyses	17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses			p. 132-154
Discussion					
Key results	18	Summarise key results with reference to study objectives			p. 155
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and		RECORD 19.1: Discuss the implications of using data that were not created or collected to answer the specific research question(s). Include discussion of misclassification bias, unmeasured confounding, missing data, and changing	p. 157-158

		magnitude of any potential bias		eligibility over time, as they pertain to the study being reported.	
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence			p. 155-157
Generalisability	21	Discuss the generalisability (external validity) of the study results			N/A
Other Information					
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based			p. 17
Accessibility of protocol, raw data, and programming code		..		RECORD 22.1: Authors should provide information on how to access any supplemental information such as the study protocol, raw data, or programming code.	p.108

A14. Vaccine effectiveness for laboratory-confirmed influenza (sub)types by season, Scotland, 2003-10

Dominant circulating strain(s) for each season	Influenza types & subtypes	Influenza-positive (cases)		Influenza-negative (controls)		Total positive (%)	Unadjusted vaccine effectiveness[a] (95% CI)	Adjusted vaccine Effectiveness[b] (95% CI)
		Vaccinated/ total (n)	Vaccinated (%)	Vaccinated/ total (n)	Vaccinated (%)			
Season: 2003-2004 A/Fujian/411/2002 (H3N2)	Influenza A & B	1/15	6.67	24/108	22.22	12.20	54.39 (-304.59 to 94.86)	34.85 (-599.09 to 93.93)
	Influenza A	1/15	6.67	24/108	22.22	12.20	54.39 (-304.59 to 94.86)	34.85 (-599.09 to 93.93)
	A(H3)	0/0	0.00	25/123	20.33	0.00	0.00 (-Inf to 100)	0.00 (-Inf to 100)
	A(H1N1)	0/0	0.00	25/123	20.33	0.00	0.00 (-Inf to 100)	0.00 (-Inf to 100)
	Influenza B	0/0	0.00	25/123	20.33	0.00	0.00 (-Inf to 100)	0.00 (-Inf to 100)
Season: 2004-2005 A/Wellington/01/2004 (H3N2) B/Shanghai/361/2002	Influenza A & B	4/16	25.00	24/83	28.92	16.16	36.23 (-145.58 to 83.44)	27.43 (-201.04 to 82.50)
	Influenza A	4/13	30.77	24/86	27.91	13.13	5.77 (-288.05 to 77.12)	0.75 (-353.41 to 78.27)
	A(H3)	0/0	0.00	28/99	28.28	0.00	0.00 (-Inf to 100)	0.00 (-Inf to 100)
	A(H1N1)	0/0	0.00	28/99	28.28	0.00	0.00 (-Inf to 100)	0.00 (-Inf to 100)
	Influenza B	0/3	0.00	28/96	29.17	3.03	100 (-Inf to 100)	100 (-Inf to 100)
Season: 2005-2006 B/Malaysia/2506/2004	Influenza A & B	2/11	18.18	28/79	35.44	12.22	73.96 (-44.80 to 95.32)	-46.01 (-1286.22 to 84.62)
	Influenza A	0/1	0.00	30/89	33.71	1.11	100 (-Inf to 100)	100 (-Inf to 100)
	A(H3)	0/1	0.00	30/89	33.71	1.11	100 (-Inf to 100)	100 (-Inf to 100)
	A(H1N1)	0/0	0.00	30/90	33.33	0.00	0.00 (-Inf to 100)	0.00 (-Inf to 100)

	Influenza B	2/10	20.00	28/80	35.00	11.11	70.21 (-70.97 to 94.81)	-69.72 (-1454.97 to 81.47)
Season: 2006-2007 A/Wisconsin/67/05 (H3N2)	Influenza A & B	2/7	28.57	15/68	22.06	9.33	16.67 (-513.54 to 88.68)	0.00 (-Inf to 100)
	Influenza A	2/7	28.57	15/68	22.06	9.33	16.67 (-513.54 to 88.68)	0.00 (-Inf to 100)
	A(H3)	2/7	28.57	15/68	22.06	9.33	16.67 (-513.54 to 88.68)	0.00 (-Inf to 10)
	A(H1N1)	0/0	0.00	17/75	22.67	0.00	0.00 (-Inf to 100)	0.00 (-Inf to 100)
	Influenza B	0/0	0.00	17/75	22.67	0.00	0.00 (-Inf to 100)	0.00 (-Inf to 100)
Season: 2007-2008 A/Solomon Island/3/2006 (H1N1)	Influenza A & B	1/9	11.11	19/78	24.36	10.34	77.14 (-118.07 to 97.60)	76.29 (-170.48 to 97.92)
	Influenza A	0/4	0.00	20/83	24.10	4.60	100 (-Inf to 100)	100 (-Inf to 100)
	A(H3)	0/1	0.00	20/86	23.26	1.15	100 (-Inf to 100)	0.00 (-Inf to 100)
	A(H1N1)	0/0	0.00	20/87	22.99	0.00	0.00 (-Inf to 100)	0.00 (-Inf to 100)
	Influenza B	1/5	20.00	19/82	23.17	5.75	75.09 (-333.05 to 98.57)	76.66 (-315.16 to 98.69)
Season: 2008-2009 A/Brisbane/10/2007 (H3N2)	Influenza A & B	18/50	36.00	81/274	29.56	15.43	-57.04 (-207.24 to 19.73)	-227.70 (-667.40 to -39.94)
	Influenza A	18/50	36.00	81/274	29.56	15.43	-57.04 (-207.24 to 19.73)	-227.70 (-667.40 to -39.94)
	A(H3)	1/11	9.10	98/313	31.31	3.40	46.21 (-519.68 to 95.33)	0.00 (-Inf to 100)
	A(H1N1)	17/36	47.22	82/288	28.47	11.11	-101.77 (-320.39 to 3.16)	-225.55 (-692.29 to -33.77)
	Influenza B	0/0	0.00	99/324	30.56	0.00	0.00	0.00

							(-Inf to 100)	(-Inf to 100)

Abbreviations: CI: confidence interval; NA: not applicable
a Adjusted for time (i.e. days) only
b Adjusted for time (i.e. days), age, number of risk groups, swab location (i.e. GP or hospital)
-No subgroup analyses are provided for the seasons 2000/01 to 2002/03 as there were not enough numbers even for overall VE estimates
-From 2003/04 to 2008/09 only VE adjusted for time and age as too few number to adjust for more variables
-There are cases with unknown influenza A subtype for all the seasons (2003-2015) which explains the total influenza A(H3) and A(H1N1) samples do not add exactly to the total influenza A samples

A15. Number of medications prescribed for asthma patients during 15 influenza seasons (total: 8,006,171, prescriptions)

Influenza season	Number of prescriptions
2002/03	173,449
2003/04	288,570
2004/05	317,128
2005/06	344,674
2006/07	376,060
2007/08	406,136
2008/09	445,776
2009/2010	464,902
2010/2011	477,443
2011/2012	500,580
2012/2013	649,187
2013/2014	958,234
2014/2015	966,116
2015/2016	985,331
2016/2017	652,585

A16. Number of asthma-related prescriptions in asthma patients (total: 4,571,904 prescriptions)

Type of asthma-related medications	Number of prescriptions
Bronchodilators	2,653,427
Inhaled corticosteroids	1,676,274
Oral corticosteroids	242,203

A17. Number of prescriptions in each of the four BTS steps (total: 8,006,171 prescriptions)

BTS steps	Number of prescriptions (% total)
Step 0	1,936,231 (24.18)
Step 1	2,822,827 (35.26)
Step 2	1,158,293 (14.47)
Step 3	2,088,820 (26.09)
Step 0: no other step; Step 1: no medium or high dose of ICS; Step 2: medium or high dose of ICS; Step 3: step 2 & OCS or OCS only or OCS & step 0 or OCS & step 1	

A18. Odds Ratio and 95% Confidence intervals of a person being swabbed for ILI in the asthma population compared to a person of the same age and sex in the general population (n=20,746,367 patient-seasons in 2010/11 in Scotland)

Predictor	Odds Ratio	95% Confidence Intervals
Asthma	1.76*	1.60 to 1.94
Age		
0-10	NA	NA
11-20	0.22*	0.18 to 0.26
21-30	0.20*	0.17 to 0.23
31-40	0.17*	0.14 to 0.20
41-50	0.19*	0.16 to 0.22
51-60	0.19*	0.16 to 0.23
61-70	0.27*	0.23 to 0.32
≥71	0.27*	0.23 to 0.31
Sex		
Female	NA	NA
Male	1.20*	1.08 to 1.33
Age*Sex		
0-10*Male	NA	NA
11-20*Male	0.63*	0.49 to 0.81
21-30*Male	0.41*	0.31 to 0.54
31-40*Male	0.71*	0.55 to 0.91
41-50*Male	0.73*	0.58 to 0.92
51-60*Male	0.93	0.73 to 1.18
61-70*Male	0.90	0.71 to 1.12
≥71*Male	1.24	1.00 to 1.53

A19. Odds Ratio and 95% Confidence intervals of a person being infected with RT-PCR laboratory-confirmed influenza in the asthma population compared to a person of the same age, sex and socioeconomic status in the general population (n=44,482 swab samples from unvaccinated individuals from 2000/01 to 2015/16 in Scotland)

Predictor	Odds Ratio	95% Confidence Intervals
Asthma	1.28*	1.17 to 1.41
Age		
0-10	NA	NA
11-20	1.43*	1.23 to 1.66
21-30	1.98*	1.73 to 1.27
31-40	1.63*	1.41 to 1.87
41-50	1.43*	1.23 to 1.65
51-60	1.31 *	1.12 to 1.52
61-70	1.14	0.96 to 1.36
≥71	1.22*	1.04 to 1.42
Sex		
Female	NA	NA
Male	1.03	0.93 to 1.14
Socioeconomic Status (SIMD)		
SIMD1 (most deprived)	1.08	0.98 to 1.19
SIMD2	1.17*	1.06 to 1.28
SIMD3	1.13*	1.02 to 1.24
SIMD4	1.16*	1.05 to 1.28
SIMD5 (least deprived)	NA	NA
Age*Sex		
0-10*Male	NA	NA
11-20*Male	1.31*	1.06 to 1.62
21-30*Male	0.90	0.73 to 1.11
31-40*Male	1.14	0.92 to 1.40
41-50*Male	1.24*	1.01 to 1.52
51-60*Male	1.25*	1.01 to 1.54
61-70*Male	0.87	0.67 to 1.13
≥71*Male	0.91	0.72 to 1.15
SIMD: Scottish Index of Multiple Deprivation; NA: not applicable		

A20. Contribution to science

Publications

- Vasileiou E, Sheikh A, Butler C, von Wissmann B, McMenamic J, Ritchie L, Tian L, Simpson C. Effectiveness of influenza vaccination for preventing influenza-related complications in people with asthma: a systematic review protocol. BMJ Open 2016; 6: e010133.
- Vasileiou E, Sheikh A, Butler C, El Ferkh K, von Wissmann B, McMenamin J, Ritchie L, Schwarze J, Papadopoulos NG, Johnston SL, Tian L, Simpson C. Effectiveness of influenza vaccines in asthma: a systematic review and meta-analysis. Clin Infect Dis 2017; 65(8): 1388-95.

Conference presentations

- Seasonal influenza vaccine effectiveness for the prevention of laboratory-confirmed influenza in a Scottish asthma population during the influenza seasons 2010-11 to 2015-16, Annual Scientific Meeting, AUKCAR, Bristol, (oral presentation), Jan. 2018.
- Effectiveness of influenza vaccines in asthma: a systematic review and meta-analysis, AUKCAR – MRC Asthma UK Joint Meeting, London (oral presentation), Sep. 2017.
- Effectiveness of influenza vaccines in asthma: a systematic review and meta-analysis, I-MOVE+ Steering-Scientific Committee and 10th Annual Meeting, Veyrier-du- Lac, France, (oral presentation), May 2017.
- Effectiveness of influenza vaccines in asthma: a systematic review and meta-analysis, AUKCAR Annual Scientific Meeting, University of Edinburgh (oral presentation), Dec. 2016.